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APPLICATION NUMBER: *60/473,806*
FILING DATE: *May 28, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/16647*

Certified by

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05/28/03

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EJ697626318

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Frank L. Zhijun Eugene A.	GREENWAY LIU WOLTERING	Baton Rouge, Louisiana Houston, Texas Kenner, Louisiana

☐ *Additional inventors are being named on th____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Inhibition of Angiogenesis and Destruction of Angiogenic Vessels with Certain Plant Extracts, Gallic Acid, and Its Derivatives

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification *Number of Pages*	35	☐ CD(s), Number	
☒ Drawing(s) *Number of Sheets*	27	☒ Other (specify)	Deposit Acct. Authorization (2 original)
☒ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.		FILING FEE AMOUNT ($)
☒ A check or money order is enclosed to cover the filing fees		
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Bonnie J. Davis

Date May 28, 2003

TYPED or PRINTED NAME Bonnie J. Davis

REGISTRATION NO. *(if appropriate)* 41,699

TELEPHONE (225) 387-3221

Docket Number: 02P01 Greenway

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT $80.00

Complete if Known	
Application Number	60/_____
Filing Date	May 28, 2003
First Named Inventor	Frank L. Greenway
Examiner Name	
Group Art Unit	
Attorney Docket No.	02P01 Greenway

METHOD OF PAYMENT *(check all that apply)*

☒ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 20-0096

Deposit Account Name: Taylor Porter Brooks & Phillips

The Commissioner is authorized to: *(check all that apply)*

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☒ Charge any additional fee(s) during the pendency of this application

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing	
1005	160	2005	80	Provisional filing fee	$80.00
				SUBTOTAL (1)	$80.00

2. EXTRA CLAIM FEES FOR UTILITY AND

	Extra Claims			Fee from below	Fee Paid
Total Claims		-20** =	0	X =	0.00
Independent Claims		- 3** =	0	X =	0.00
Multiple Dependent				=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent
				SUBTOTAL (2) $0.00

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

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1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non - English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR § 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Statement	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
Other fee (specify) _____					

*Reduced by Basic Filing Fee Paid SUBTOTAL (3)

SUBMITTED BY

				Complete *(if applicable)*	
Name *(Print/Type)*	Bonnie J. Davis	Registration No. *(Attorney/Agent)*	41,699	Telephone	(225) 387-3221
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Express Mail No. EJ697626318

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Frank L. Greenway *et al.*
Serial Number:	60/_____
Filing Date:	May 28, 2003
Title:	Inhibition of Angiogenesis and Destruction of Angiogenic Vessels with Certain Plant Extracts, Gallic Acid, and Its Derivatives
Attorney File:	02P01 Greenway

Mail Stop Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
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DEPOSIT ACCOUNT AUTHORIZATION AND REQUEST FOR AUTOMATIC EXTENSIONS OF TIME

Dear Sir:

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17, and 1.492 that may be required during the entire pendency of this application, and to credit any amounts paid in excess of those actually due, to Deposit Account No. 20-0096.

It is applicants' intention generally to pay all fees required under 37 C.F.R. §§ 1.16, 1.17, and 1.492 by timely-submitted checks. However, in those instances where no check is timely submitted, or where a check is submitted for an amount less than the amount required, the Commissioner is authorized to charge any fees under 37 C.F.R. §§ 1.16,

Express Mail No. EJ697626318

1.17, and 1.492 that may therefore be required during the entire pendency of this application to Deposit Account No. 20-0096.

Pursuant to 37 C.F.R. § 1.136(a)(3), please treat this paper as a request to treat any concurrent or future replies filed in this application, that require a petition for an extension of time under 37 C.F.R. § 1.136(a) for timely submission, as incorporating a petition for an extension of time for the appropriate length of time. Where the necessary fees for an extension of time are not paid by contemporaneously-submitted check, please charge Deposit Account No. 20-0096 as discussed above.

Respectfully submitted,

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May 28, 2003

Application Data Sheet

Application Information

Application Type::	Provisional
Subject Matter::	Utility
Title::	Inhibition of Angiogenesis and Destruction of Angiogenic Vessels with Certain Plant Extracts, Gallic Acid, and Its Derivatives
Attorney Docket Number::	02P01 Greenway
Total Drawing Sheets::	27
Small Entity?::	Yes

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PROVISIONAL PATENT APPLICATION

INHIBITION OF ANGIOGENESIS AND DESTRUCTION OF ANGIOGENIC VESSELS WITH CERTAIN PLANT EXTRACTS, GALLIC ACID, AND ITS DERIVATIVES

Frank L. Greenway, Zhijun Liu, and Eugene A. Woltering

Express Mail No. EJ697626318
File No. 02P01 Greenway

[0001] The development of this invention was subject to a contract between the Board of Supervisors of Louisiana State University and Agricultural and Mechanical College, and the United States Department of Veterans Affairs. The United States Government has certain rights in this invention.

[0002] This invention pertains to a method to inhibit angiogenesis by use of extracts of certain *Rubus* plant species and of certain other plants, and by use of gallic acid and its active derivatives.

Angiogenesis

[0003] In an adult, two types of blood vessels can potentially be found. The normal blood vessel is a resting, quiescent, fully developed vessel. A second form, a proliferating or developing blood vessel, occurs rarely during the normal life cycle (only in early development and reproduction, e.g., menstrual cycle and pregnancy). In contrast, the process of angiogenesis, the proliferation and development of new blood vessels, often occurs in wound healing and in pathological processes, e.g., tumor growth. Angiogenesis is a complex process involving many stages, including extracellular matrix remodeling, endothelial cell migration and proliferation, capillary differentiation, and anastomosis. All detectable solid tumors (tumors over 2mm in diameter) exploit angiogenesis to supply the needed blood to proliferating tumor cells. Studies have demonstrated that the level of vascularization in a tumor is strongly associated with metastasis in melanoma, breast, and lung

carcinomas. See R. Bicknell, "Vascular targeting and the inhibition of angiogenesis," Annals of Oncology, vol. 5, pp. 45-50 (1994).

[0004] Angiogenesis inhibitors have been suggested to intervene into neoplastic processes. See G. Gasparini, "The rationale and future potential of angiogenesis inhibitors in neoplasia," Drugs, vol. 58, pp. 17-38 (1999). The inhibitory agents block angiogenesis, thereby causing tumor regression in various types of neoplasia. Known therapeutic candidates include naturally occurring angiogenic inhibitors (e.g., angiostatin, endostatin, platelet factor-4), specific inhibitors of endothelial cell growth (e.g., TNP-470, thalidomide, interleukin-12), agents that neutralize angiogenic molecules (e.g., antibodies to fibroblast growth factor or vascular endothelial growth factor), suramin and its analogs, tecogalan, agents that neutralize receptors for angiogenic factors, agents that interfere with vascular basement membrane and extracellular matrix (e.g., metalloprotease inhibitors, angiostatic steroids), and anti-adhesion molecules (e.g., antibodies such as anti-integrin alpha v beta 3). See L. Rosen, "Antiangiogenic strategies and agents in clinical trials," Oncologist, vol. 5, supplement 1, pp. 20-27 (2000).

[0005] Abnormal angiogenesis occurs when improper control of angiogenesis causes either excessive or insufficient blood vessel growth. Excessive blood vessel proliferation favors tumor growth and development of distant metastases, blindness, skin disorders such as psoriasis, and rheumatoid arthritis. Diseases that have been associated with neovascularization include, for example, Crohn's disease, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoidosis, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme disease, systemic lupus erythematosis, psoriasis, retinopathy of prematurity, Eales disease, Bechets disease, infections causing retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndrome, toxoplasmosis, trauma, rheumatoid arthritis, and post-laser complications. Other angiogenic-related diseases may include, for example, diseases associated with rubeosis (neovascularization of the angle), and diseases caused by abnormal proliferation of fibrovascular or fibrous tissue, including all forms of proliferative vitreoretinopathy. Any disease having a known angiogenic counterpart could

potentially be treated with an anti-angiogenic factor, e.g., psoriasis. See D. Creamer *et al.*, "Overexpression of the angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase in psoriatic epidermis," Br. J. Dermatol., vol. 137, pp. 851-855 (1997).

[0006] Angiogenesis is a prominent contributor to solid tumor growth and the formation of distant metastases. Several experimental studies have concluded that primary tumor growth, tumor invasiveness, and metastasis all require neovascularization. The process of tumor growth and metastasis is complex, involving interactions among transformed neoplastic cells, resident tissue cells (e.g., fibroblasts, macrophages, and endothelial cells), and recruited circulating cells (e.g., platelets, neutrophils, monocytes, and lymphocytes). A possible mechanism for the maintenance of tumor growth is an imbalance, or disregulation, of stimulatory and inhibitory growth factors in and around the tumor. Disregulation of multiple systems allows the perpetuation of tumor growth and eventual metastasis. Angiogenesis is one of many systems that is disregulated in tumor growth. In the past it has been difficult to distinguish between disregulation of angiogenesis and disregulation of other systems affecting a developing tumor. Another complicating factor is that aggressive human melanomas mimic vasculogenesis by producing channels of patterned networks of interconnected loops of extracellular matrix, in which red blood cells, but not endothelial cells, are detected. See A.J. Maniotis *et al.*, "Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: Vasculogenic mimicry," Am. J. Pathol., vol. 155, pp. 739-52 (1999). These channels may facilitate perfusion of tumors, independent of perfusion from angiogenesis.

[0007] A tumor cannot expand beyond approximately 2 mm without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors including acoustic neuroma, neurofibroma, trachoma, and pyogenic granulomas. Inhibiting angiogenesis could halt the growth and potentially lead to regression of these tumors. Angiogenic factors have been reported as being associated with several solid tumors, including rhabdomyosarcoma, retinoblastoma, Ewing sarcoma, neuroblastoma, and osteosarcoma.

[0008] Angiogenesis has also been associated with some non-solid tumors, including blood-born tumors such as leukemias, various acute or chronic neoplastic diseases of the bone marrow marked by unrestrained proliferation of white blood cells, usually accompanied by anemia, impaired blood

clotting, and enlargement of the lymph nodes, liver, and spleen. It is believed that angiogenesis may play a role in the abnormalities in the bone marrow that give rise to leukemias and multiple myelomas.

[0009] Antiangiogenic factors inhibit tumor growth beyond 2 mm by inhibiting the angiogenic response and thus inhibiting blood vessel growth to the tumor. Although angiogenesis in a tumor may begin at an early stage, a tumor requires a blood supply to grow much beyond about 2 mm. Up to 2 mm diameter, tumors can survive by obtaining nutrients and oxygen by simple diffusion. Most anti-angiogenic factors are not cytotoxic, i.e., capable of killing the tumor cells directly. Small tumors of a size about 1 mm^3 can be effectively inhibited and destroyed by factors, either endogenous or exogenous, that stimulate the immune system. It is generally accepted that once a tumor has reached a critical size, the immunological system is no longer able to effectively destroy the tumor; i.e., there is a negative correlation between tumor size and immune competence. See A.K. Eerola *et al.*, "Tumour infiltrating lymphocytes in relation to tumour angiogenesis, apoptosis," Lung Cancer, vol. 26, pp. 73-83 (1999); and F.A. Wenger *et al.*, "Tumor size and lymph-node status in pancreatic carcinoma -- is there a correlation to the preoperative immune function?," Langenbecks Archives of Surgery, vol. 384, pp. 473-478 (1999). Early adjuvant use of an effective anti-angiogenic agent to preclude development of tumor metastases beyond 1 to 2 mm^3 may allow more effective tumor attack and control by the body's immunological mechanisms. In addition, prolonged adjuvant use of a non-toxic angiogenic inhibitor may prevent tumor dissemination by blocking the growth of vessels required for the transport of tumor cells that would form metastatic foci.

[0010] Angiogenesis has also been implicated in obesity. Mice treated with anti-angiogenic agents lost weight and adipose tissue. See M.A. Pupnick *et al.*, "Adipose tissue mass can be regulated through the vasculature," PNAS, vol. 99, pp. 10730-10735 (2002).

[0011] New antiangiogenic factors are needed, in particular, compounds that not only inhibit new angiogenic growth, but also that degrade existing capillary networks. Very few antiangiogenic factors have been reported to diminish existing capillary networks.

Chinese Blackberry,* Rubus suavissimus *S. lee

[0012] *Rubus suavissimus* S. Lee, a perennial shrub, Chinese blackberry, is one of some 62 species in the genus *Rubus* of the Rosaceae family. It is widely distributed in the southwest of China but flourishes in Guangxi Autonomous Region. Leaves of Chinese blackberry have long been used in southern China as a tea due to its sweet taste, thus the Chinese name Tiancha or Sweet Leaf Tea. The sweet taste is due to the presence of dipterpene glucosides in the leaves, one of which is rubusoside, reaching a concentration of over 5% (w/w). See T. Tanaka *et al.*, "Rubusoside (β-D-glucosyl ester of 13-*O*-β-D-glucosyl-steviol), a sweet principle of *Rubus chingii* Hu (Rosaceae)," Agric. Biol. Chem., vol. 45, pp. 2165-2166 (1981); and T. Seto *et al.*, "β-Glucosyl esters of 19α-hydroxyursolic acid derivatives in leaves of *Rubus* species," Phytochemistry, vol. 23, pp. 2829-2834 (1984). There were other diterpene glucosides found in the leaves, e.g., suavioside A and suaviosides B, C_1, D_2, F, G, H, I, and J. See S. Hirono *et al.*, "Sweet and bitter diterpene-glucosides from leaves of *Rubus suavissimus*," Chem. Pharm. Bull., vol. 38, pp. 1743-1744 (1990); W.-H. Zhou *et al.*, "A new sweet diterpene-glucoside in leaves of *Rubus suavissimus*, "" Acta Botanica Sinica, vol. 34, pp. 315-318 (1992); and K. Ohtani *et al.*, "Minor diterpene glycosides from sweet leaves of *Rubus suavissimus*," Phytochemistry, vol. 31, pp. 1553-1559 (1992). Further chemical analyses over the leaves of thirty-nine other *Rubus* spp. revealed that the presence of diterpene glycosides is only limited to the leaves of *R. suavissimus* and *R. chingii*, whereas glucosyl 19α-hydroxyuresana-type triterpenes are more common as constituents in the leaves of *Rubus* spp. See F. Gao *et al.*, "19α-hydroxyursane-type triterpene glucosyl esters from the roots of *Rubus suavissimus* S. Lee," Chem. Pharm. Bull., vol. 33, pp. 37-40 (1985)

[0013] In southern China, especially in Guangxi Autonomous Region, the leaves of *R. suavissimus* are used not only as tea and a food additive, but also as herbal medicines thought to nourish the kidneys and lower blood pressure. See P.-F. Huang *et al.*, "Comprehensive utilization of *Rubus suavissimus* S. Lee," Guangxi Huagong, vol. 31, pp. 24-25 (2002). The leaf of Chinese blackberry has also been said to help with fever, to relieve stress on the lungs, to reduce the secretion of phlegm, and to relieve coughs. See Y. Ono, "The health beneficial effects of Tien-cha (*Rubus suavissimus* tea) and its applications," Food Style 21, vol. 6, pp. 77-80 (2002). Recent studies

indicated an anti-inflammatory and anti-allergy effect. See U. Kotaro, "Antiallergy action of *Rubus suavissimus*," Shokuhin Kogyo, vol. 40, pp. 52-59 (1997); K. Nakahara, "Anti-allergic activity of Tiencha and oolong tea polyphenols," Food Style 21, vol. 2, pp. 45-49 (1998); and K. Nakahara *et al.*, "Anti-allergic composition containing GOD-type ellagitannin as active ingredient," European Patent Application No. 727218 (1996).

Gallic Acid

[0014] Gallic acid or 3, 4, 5-trihydroxy benzoic acid, is a colorless crystalline organic acid found in many plants. The list of plants that have been shown to contain gallic acid include: *Abrus prccatorius L.*; *Acacia catechu (L.) Willd.*; *Ampelopsis brevipedunculata*; *Ampelopsis japonica*; *Coriaria sinica Maxim.*; *Cornus officinalis Sieb. et Zucc.*; *Cotinus coggygria Scop.* (Smokebush); *Daucus carota L. var. Sativa DC.*; *Erodium stephanianum Willd.*; *Eucalyptus robusta Sm.*; *Euonymus bungeanus Maxim.* (Winterberry Euonymus); *Euphorbia humifusa Wild.* (Wolf's milk); *Geranium pratense L.*; *Geranium wilfordii Maxim.* (Heron's Bill); *Juglans regia L.*; *Loropetalum chinensis (R. Br.) Oliv.* (Chinese fringe tree); *Lythrum salicaria L.*; *Malus* spp. (Apple); *Mangifera indica L.* (Mango); *Macrocarpium officinale Sieb. et Zucc.*; *Passiflora caerulea L.*; *Pharbitis nil (L.) Choisy*; *Phyllanthus emblica L.*; *Pistacia chinensis Bge.*; *Platycarya longipes Wu.*; *Platycarya strobilacea Sieb. et Zucc.* (Australia cheesewood); *Polygonum aviculare L.*; *Polygonum bistorta l.* (Bistort); *Psidium guajava L.*; *Quercus infectoria Oliver*; *Rheum officinale Baill.*; *Rheum palmatum L.*; *Rheum tanguticum Maxim. Ex Reg.*; *Rhus chinensis Mill.* (Chinese sumac gallnut); *Rhus potaninii Maxim.* (Sumac gallnut); *Rosa chinensis Jacq.* (Mini rose); *Rosa rugosa Thunb.* (Rose); *Rubus ulmifolius*; *Rumex japonicus Houtt.* (Japanese dock); *Sanguisorba officinalis L.* (Burnet); *Sapium sebiferum (L.) Roxb.*; *Syzygium cumini (L.) Skeels*; *Tamarix chinensis Lour.*; *Terminalia chebula Retz.* (Medicine terminalia); *Tetrastigma hypoglaucum Planch.*; and *Tussilago farfara L.*. See U.S. Patent No. 6,444,236; *Colored Illustrations of Chinese Traditional and Herbal Ordinary Drugs in China*, Wu Jianrong and Qiu Dewen, editors; Guizhou Technology and Science Press, Guiyang, China (1993); Z. Liu *et al.*, *Encyclopedia of Woody Medicinal Plants of China*, CD-ROM, Academic Services Associates, Inc., Seattle, Washington (2000); D. Liu *et al.*, "Studies on Chemical

Constituents from Tetrastigma Hypoglaucum," Chinese Trad. And Herbal Drugs, vol. 34, pp. 4-6 (2003); L. Panizzi *et al.*, "In Vitro Antimicrobial Activity of Extracts and Isolated Constituents of Rubus Ulmifolins," J. Ethnopharmacol., vol. 29, pp. 165-8 (2002); Encyclopedia of Traditional Chinese Medicine, Shanghai S&T Press (1986); and K. Wolfe *et al.*, "Antioxidant activity of apple peels," J. Agric. Food Chem., vol. 51, pp. 609-14 (2003).

[0015] Since gallic acid has hydroxyl groups and a carboxylic acid group in the same molecule, two molecules can react to form an ester, digallic acid. Gallic acid is usually obtained by the hydrolysis of tannic acid with sulfuric acid. Gallic acid is known to be a strong natural antioxidant. See K. Polewski *et al.*, "Gallic acid, a natural antioxidant, in aqueous and micellar environment: spectroscopic studies," Current Topics in Biophysics, vol. 26, pp. 217-227 (2002).

[0016] Gallic acid is wide-spread in plant foods and beverages such as tea and wine and has been shown to be one of the anticarcinogenic polyphenols present in green tea. Gallic acid has been shown to display selective cytotoxicity against tumor cells, and to induce apoptosis in tumor cells. See K. Isuzugawa *et al.*, "Different generation of inhibitors against gallic acid-induced apoptosis produces different sensitivity to gallic acid," Biol. Pharm. Bull., vol. 24, pp. 249-253 (2001). Also, theaflavin monogallates and digallates isolated from tea have been shown to inhibit cancer cell growth and induce apoptosis. See, e.g., J. Lu *et al.*, "Differential effects of theaflavin monogallates on cell growth, apoptosis, and *Cox-2* gene expression in cancerous versus normal cells," Cancer Research, vol. 60, pp. 6465-6471 (2000); T. Ohno *et al.*, "Cytotoxic activity of gallic acid against liver metasis of mastocytoma cells P-815," Anticancer Res., vol. 21, pp. 3875-80 (2001); and G.Y. Yang *et al.*, "Effect of black and green tea polyphenols on c-jun phosphorylation and H_2O_2 production in transformed and non-transformed human bronchial cell lines: possible mechanisms of cell growth inhibition and apoptosis induction," Carcinogenesis, vol. 21, pp. 2035-2039 (2000). The anti-tumor promoting active constituents of the fruits of *Caesalpinia ferrea* were identified as gallic acid and methyl gallate. See E.S. Nakamura *et al.*, "Cancer chemopreventive effects of constituents of Caesalpinia ferrea and related compounds," Cancer Lett., vol. 177, pp. 119-24 (2002). Orally administered gallic acid, with and without the anti-cancer drug cisplatin, was found to cause

apoptosis in lung cancer cells transplanted in mice. See M. Kawada, "Anti-tumor effect of gallic acid on LL-2 lung cancer cells transplanted in mice," Anticancer Drugs, vol. 12, pp. 847-852 (2001).

[0017] U.S. Patent Application No. 2002/0068094 discloses a physiologically active extract from indigo which includes tryptanthrin, 3,5,4'-rihydroxy-6,7-m-ethylenedioxy-flavone, kaempferol, 3,5,7,4'-tetrahydroxy-6-methoxy-flavone, gallic acid, caffeic acid, indirubin, pheophorbide a, and methyl pheophorbide a. Although indicating that the extract may have many different physiological functions, experiments are discussed only to show antiseptic action, antiviral action, antitumor action, radical-entrapping action, apoptosis controlling action, and action for controlling the production of cytokine. Gallic acid was shown to have radical-entrapping action.

[0018] We have discovered that an extract of Chinese blackberry (*Rubus suavissimus*) inhibited angiogenesis. From the extract, a least two fractions were isolated that showed powerful anti-angiogenic activity. From one of these fractions, gallic acid was shown to be the active compound. The antiangiogenic activity was measured by an assay that is an *in vitro* human angiogenesis model using a human placental vein disc. Extracts from other plants either known or found to have gallic acid, e.g., rhubarb root, persimmon fruit, blackberry (*Rubus fruticosus*) leaf and berry, and dogwood berry, were also found to have anti-angiogenic activity. Gallo tannin (tannic acid) was also found to inhibit angiogenesis. Other derivatives of gallic acid will be tested for their anti-angiogenic activity, including methyl gallate, ethyl gallate, propyl gallate, butyl gallate, lauryl gallate, octyl gallate, ellagic acid, BUSMUTH-gallate, galloyl glucose, di-galloyl glucose, tri-galloyl glucose, tetra-galloyl glucose, and penta-galloyl glucose. The extract from Chinese blackberry also slowed the growth of a pancreatic tumor and of corneal neovascularization in rats. Extracts from *Rubus* spp, and other plants with gallic acid, and gallic acid will be useful for treating various diseases associated with neovascularization, including diabetic retinopathy, psoriasis, tumors, obesity, cancer, rheumatoid arthritis, etc.

Brief Description of the Drawings

[0019] Fig. 1 illustrates the effect of 0.1% Chinese blackberry leaf extract (RUS) on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0020] Fig. 2a illustrates the effect of Chinese blackberry leaf extract (RUS) at various concentrations on the initiation of angiogenesis in human placental vein discs.

[0021] Fig. 2b illustrates the effect of Chinese blackberry leaf extract (RUS) at various concentrations on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0022] Fig. 2c illustrates the effect of Chinese blackberry leaf extract (RUS) at various concentrations on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0023] Fig. 3a illustrates the effect of Chinese blackberry leaf extract (RUS) after subjected to various treatments and reconstituted to 0.1% (w/v) on the initiation of angiogenesis in human placental vein discs.

[0024] Fig. 3b illustrates the effect of Chinese blackberry leaf extract (RUS) after subjected to various treatments and reconstituted to 0.1% (w/v) on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0025] Fig. 3c illustrates the effect of Chinese blackberry leaf extract (RUS) after subjected to various treatments and reconstituted to 0.1% (w/v) on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0026] Fig. 4a illustrates the effects of several fractions from a Chinese blackberry leaf extract (RUS-F) at a 0.1% concentration on the initiation of angiogenesis in human placental vein discs.

[0027] Fig. 4b illustrates the effects of several fractions from a Chinese blackberry leaf extract (RUS-F) at a 0.1% concentration on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0028] Fig. 4c illustrates the effects of several fractions from a Chinese blackberry leaf extract (RUS-F) at a 0.1% concentration on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0029] Fig. 5a illustrates the effect of a purified compound isolated from the leaf extract of Chinese blackberry (0.025% RUSF260) on the initiation of angiogenesis in human placental vein discs.

[0030] Fig. 5b illustrates the effect of a purified compound isolated from the leaf extract of Chinese blackberry (0.025% RUSF260) on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0031] Fig. 5c illustrates the effect of a purified compound isolated from the leaf extract of Chinese blackberry (0.025% RUSF260) on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0032] Fig. 6a illustrates the effect of several concentrations of gallic acid on the initiation of angiogenesis in human placental vein discs.

[0033] Fig. 6b illustrates the effect of several concentrations of gallic acid on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0034] Fig. 6c illustrates the effect of several concentrations of gallic acid on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0035] Fig. 7a illustrates the effect of extracts from several plants known or found to contain gallic acid on the initiation of angiogenesis in human placental vein discs.

[0036] Fig. 7b illustrates the effect of extracts from several plants known or found to contain gallic acid on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0037] Fig. 7c illustrates the effect of extracts from several plants known or found to contain gallic acid on angiogenesis in human placental vein discs as measured by an angiogenic index after

removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0038] Fig. 8a illustrates the effect of tannic acid (a derivative of gallic acid) on the initiation of angiogenesis in human placental vein discs.

[0039] Fig. 8b illustrates the effect of tannic acid (a derivative of gallic acid) on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0040] Fig. 8c illustrates the effect of tannic acid (a derivative of gallic acid) on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

[0041] Fig. 9 illustrates the angiogenic activity of several serum samples isolated from rats that were given various doses of the RUS extract from *Rubus suavissimus* (Chinese blackberry) both orally and as an intraperitoneal injection (IP).

[0042] Fig. 10 illustrates the effect of a weekly intraperitoneal injection of the RUS extract (1%) on the size of pancreatic tumors in rats.

[0043] Fig. 11a illustrates the effect of extracts (0.1%) of Chinese blackberry leaves (RUS), blackberry leaves (RUF-L), and blackberry berries (RUF-F) on the initiation of angiogenesis in human placental vein discs.

[0044] Fig. 11b illustrates the effect of extracts (0.1%) of Chinese blackberry leaves (RUS), blackberry leaves (RUF-L), and blackberry berries (RUF-F) on angiogenesis (both initiation and proliferation) in human placental vein discs as measured by an angiogenic index.

[0045] Fig. 11c illustrates the effect of extracts (0.1%) of Chinese blackberry leaves (RUS), blackberry leaves (RUF-L), and blackberry berries (RUF-F) on angiogenesis in human placental vein discs as measured by an angiogenic index after removing discs with a zero angiogenic index (i.e., discs that never initiated an angiogenic response).

Example 1

Materials and Methods for Angiogenesis Assay

[0046] *The Human Placental Vein Angiogenesis Model:* Discarded human placentas were obtained anonymously with prior approval of an Institutional Review Board. The placental veins were dissected free from the placenta and adventitial tissue. The trimmed vein segment was opened longitudinally to produce a flat film of venous tissue of full thickness. Vein discs (2 mm diameter) were created with a sterile skin punch (Miltex Instrument Company, Inc.; Lake Success, New York). The discs were placed into wells of a standard 96-well plate (Corning Inc., Corning, New York). The vein disc harvest was completed within three hours of delivery to optimize endothelial cell viability. Vein discs from a single placenta were distributed equally among all treatment groups to ensure randomization. Each well was preloaded with a human thrombin solution (0.05 IU in 2.0 µl), and allowed to evaporate to dryness before use. All chemicals were purchased from Sigma Chemical Company (St. Louis, Missouri) unless otherwise indicated.

[0047] Following the placement of the 2 mm vein disc in the bottom of each thrombin-containing well, the disc was covered with 100 µl of a clot-forming medium, comprising 3 mg/ml fibrinogen and 0.5% Σ-amino caproic acid dissolved in Human Placental Vein Angiogenesis Media (HPVAM). HPVAM is made of Medium 199 (Vitrogen Corporation, Carlsbad, California), an antibiotic/antimycotic solution (100 U/ml penicillin, 100 U/ml streptomycin sulfate, and 0.25 µg/ml amphotericinβ; Vitrogen Corporation), and endothelial growth medium (25%) (Vitrogen Corporation). The mixture was allowed to clot by incubating in 5% CO_2, 95% air at 37°C in a humidified incubator. After the medium-containing placental discs had clotted, the vein-containing clot was supplemented with 100 µl HPVAM containing 20% fetal bovine serum (Vitrogen Corporation). The total well volume was 200 µl.

[0048] *Evaluation of Angiogenesis:* Visual evaluation of all wells was performed at 20X or 40X magnification with a standardized reference grid by an unbiased observer using an inverted microscope. Every other day, discs were graded using two criteria: the initiation of sprouting vessels (initiation) and the degree of sprouting (angiogenic index). Initiation of an angiogenic response was defined as the development of three or more vessel sprouts around the periphery of the

vein disc. Initiation occurred in 50 - 95% of the wells, usually 4 to 6 days after establishment of the clots. Initiation was expressed as the percent of the total wells plated that indicated an angiogenic response.

[0049] The angiogenic index (AI) was defined using a subjective visual rating system. Each disc was visually rated for the development of vessel sprouting in each of four quadrants. Each of the four quadrants for each disc was rated on a 0-4 scale, depending on the number of sprouts (density) and the length of sprouts. Scores for all four quadrants were summed to express the AI, a numerical rating that could range from 0 to 16. A score of zero indicated no vessel growth in any of the four quadrants, while a score of 16 indicated long, dense angiogenic vessel growth in all four quadrants. For most experiments, the AI was expressed as a mean plus/minus a standard error of the mean.

[0050] To separate the process of initiation from that of proliferation, the AI was analyzed both with zero AI data points and without zero AI data points. A zero AI indicated that no angiogenic initiation occurred in that disc. This lack of initiation could have been due either to the effect of the experimental compound, to the insensitivity of the vein disc to stimulation in the culture conditions, or to the vein disc not being viable. In previous experiments, we have shown that only a small percent, about 2 to 3%, of vein discs are not viable (data not shown). Thus, a graph of AI with zero AI data points indicates the complete angiogenic response of initiation and growth under the experimental conditions. However, a graph of AI without the zero AI data points indicates only growth of the vessels after initiation.

[0051] To assay for the effect of gallic acid or plant extracts on angiogenesis, the solution to be tested was added in various percentages to HPVAM to yield the tests groups. The control medium was HPVAM supplemented with a matching concentration of NaCl to ensure that the observed effects were not due to a difference in concentration of the medium ingredients. Every two days the medium in each well was replaced, and each well was scored for both initiation of angiogenesis and angiogenic index.

Example 2

Rubus suavissimus *Extractions Assay*

[0052] *RUS Extract:* The leaves of *Rubus suavissimus*, Chinese blackberry, were collected from Guangxi, People's Republic of China. The leaves were air-dried and stored at room temperature before extraction. One hundred grams of dried leaves were soaked in 2 L tap water for 1 hr and then brought to a boil two times for 30 min each. The mixture was then filtered with cheesecloth and spray dried to obtain 31 g crude extract powder, the "RUS" extract.

Treatments and Fractionation of the RUS Extract

[0053] *RUS-A Extract:* The crude extract RUS was re-constituted with deionized water and subjected to five consecutive freeze-thaw cycles to yield "RUS-A" extract.

[0054] *RUS-B Extract:* Crude extract RUS was re-constituted with deionized water and boiled for 20 min to yield "RUS-B" extract.

[0055] *RUS-C and RUS-D Extracts:* Crude extract RUS was re-constituted with deionized water, and mixed with 20% trichloroacetic acid (TCA) at a 1:1 v/v ratio and 0.1 ml 10% bovine serum albumin. This mixture was incubated at 4°C for 30 min and centrifuged at 300x for 30 min. The pH was adjusted to 7 with NaOH, and the mixture re-centrifuged. The precipitate and supernatant were separated to become RUS-C and RUS-D extracts, respectively.

[0056] *RUS-E and F Extracts:* Crude extract RUS was re-constituted with deionized water, and the mixture extracted with 0.8 volume of chloroform three times. The aqueous phase was collected and freeze-dried to yield RUS-F fraction powder. The chloroform phase was collected and further processed. The chloroform was removed by sequentially adding 200 ml methanol to facilitate the evaporation, 500 ml deionized water to remove methanol, and then another 200 ml deionized water to remove any residual organic solvents from the liquid extract. The aqueous liquid extract was then freeze-dried to powder, the "RUS-E" extract.

[0057] *Further Fractions of RUS-F:* The RUS-F fraction was fractionated using column chromatography. The RUS-F powder was dissolved in 50% methanol and then loaded on a 30 g Sephadex LH-20 column. After loading the sample, 50% methanol was used to elute the column. Each 10 ml eluate was collected during the first 100 ml of eluate for 10 fractions (Eluate 1 through

10), and then the second 100 ml eluate was collected as a whole (Eluate 11). Chemical fingerprints of each eluate were obtained using a high performance liquid chromatography system (Beckman Instruments, Fullerton, California). A C18 column (15 cm long, 4.6 mm internal diameter) was used. The mobile phase was set at 1% methanol in water and a flow rate of 1 ml per minute. UV absorption was measured at 254 nm wavelength. Eluate fractions 4 through 10 indicated similar chemical fingerprints, and were combined. The other eluates were kept separate. The resulting eluate fractions were removed of organic solvents, and freeze-dried to yield powder fractions named "RUS-F01" (eluate fraction 1), "RUS-F02" (eluate fraction 2), "RUS-F03" (eluate fraction 3), "RUS-F04" (eluate fractions 4 through 10), and "RUS-F11" (eluate fraction 11).

Example 3

Gallic Acid Extraction and Source

[0058] *Source of Gallic Acid:* Gallic acid was isolated and purified as described below from aqueous Chinese blackberry extract prepared from air-dried leaves purchased from Guangxi Botanical Garden, Nanning and Guangxi Normal University S&T New Tech Company, Guilin, China. Gallic acid, methyl gallate, ethyl gallate, propyl gallate, butyl gallate, lauryl gallate, octyl gallate, ellagic acid, BUSMUTH-gallate were purchased from Sigma Chemical Company (St. Louis, Missouri).

Extraction of Gallic Acid from Chinese Blackberry

[0059] Fraction RUS-F11 as isolated in Example 2 was further purified by dissolving in 50% methanol and again loading on a Sephadex LH-20 column. This purification step was done twice and yielded a pure compound, initially labeled "RUSF260." RUSF260 was shown to be gallic acid by several methods. The chemical structure of RUSF260 was elucidated in CD3OD carrier solvent on a Bruker DPX400 MHz Nuclear Magnetic Resonance Spectrometer using both ^{1}HNMR and ^{13}CNMR and comparing the spectra with standards. RUSF260 was determined to be gallic acid (data not shown). In addition, mass spectrometry confirmed that the molecular weight of RUSF260 (170.2) matched that of gallic acid. (data not shown)

Example 4

Extraction of Other Plant Sources

[0060] Rhubarb root (Anguo Herbal Product Market, Hebei, China; grown in Gansu, China), persimmon calyx (Southside Produce, Baton Rouge, Louisiana), and dogwood berry (Anguo Herbal Product Market, Hebei, China; grown in Henan, China) were separately ground to particles of 6 mm or smaller dimensions. For each plant, ten grams of ground particles were added to a 1 L flask with 200 ml ddH_2O and allowed to soak overnight at room temperature (20 to 25°C). The mixture was then boiled for 30 min. After cooling, the supernatant was collected and filtered through a 20 μM filter paper. The filtrate was then concentrated in a rotary evaporator, and then freeze-dried to a powder. This extraction procedure produced the following amounts of crude extracts: rhubarb, 1.98 g; persimmon, 1.27 g; and dogwood, 2.27 g.

Example 5

Inhibitory Effects of a Chinese blackberry Extract (RUS) on Angiogenesis

[0061] To test the effectiveness of Chinese blackberry extract on pre-existing angiogenesis, human placental vein discs (PVD) were obtained as above and grown for 9 days in HPVAM with the medium changed every two days. Two groups each with 9 PVDs were used for the control and for the Chinese blackberry extract. At day 9, 0.1% Chinese blackberry extract (extract RUS as named in Example 2) was added to the HPVAM when all PVDs had initiated angiogenesis. Every two to three days after the addition of the Chinese blackberry extract, the PVD were scored and the medium was changed as discussed in Example 1.

[0062] The number of wells with angiogenic vessels decreased upon addition of 0.1% Chinese blackberry extract. Fig. 1 shows the angiogenic index without the zero points which indicates the growth of angiogenic vessels after initiation as described in Example 1. Each data point represents an average of 7 observations. The x-axis in Fig. 1 represents the number of days of culture/the number of days since addition of Chinese blackberry extract. The Chinese blackberry-treated PVD stopped angiogenesis whereas untreated PVD continued to develop angiogenesis at a steady rate until day 19.

[0063] To test the effects of the RUS extract at various concentrations on angiogenesis, PVDs were grown in HPVAM supplemented with the RUS extract re-constituted with HPVAM to the following concentrations: 0.1%, 0.075%, 0.05%, 0.025%, and 0.01%. (w/v). The control group was supplemented with similar concentrations of NaCl. For each group, 20 PVDs were used. The PVDs were allowed to grow for six days in only HPVAM before adding either NaCl or the RUS extract. Then every two to three days the medium in each well was replaced, and each well scored for both initiation of angiogenesis and the angiogenic index. As shown in Fig. 2a, the initiation of angiogenesis was totally inhibited by 0.05% or higher RUS concentrations, partially inhibited by 0.025%, but was not affected by 0.01%. Similar results were seen in the angiogenic index both with and without the zero data points. (Fig. 2b and 2c) However, 0.01% RUS showed some inhibitory action in the angiogenic index, indicating some anti-proliferation activity.

[0064] These results indicate that the Chinese blackberry extract contains effective and potent anti-angiogenic compounds.

Example 6

Inhibitory Effects After Various Treatments of RUS Extract on Angiogenesis

Treatments and Initial Fractions of RUS

[0065] To identify the active anti-angiogenic compounds present in the RUS extract, the extract was subjected to various treatments and several fractions collected as described in Example 2. PVDs were grown in HPVAM supplemented with either RUS, RUS-A, RUS-B, RUS-C, RUS-D, RUS-E or RUS-F. All extracts were reconstituted to a concentration of 0.1% (w/v) in HPVAM. Two control groups were used: one group with NaCl as a supplement, and a second group with a heparin-steroid combination for a positive control. The positive control group was treated with a heparin-steroid (21-phosphate hydrocortisone) mixture (300 μg/ml and 350 μg/ml, respectively), which was previously found to reduce angiogenesis by 30 to 40%. Twenty PVDs for each group was used. The PVDs were grown for six days in only HPVAM before adding any extract or control supplement. After addition of the supplements, every two to three days, the medium was replaced in each well, and each well was scored for both initiation of angiogenesis and angiogenic index.

[0066] As shown in Fig. 3a, the initiation of angiogenesis was totally inhibited by the RUS-C and RUS-F fractions and only partially inhibited by RUS-D and RUS-E fractions. RUS-A and RUS-B, the boiling and freeze-thaw treatments of RUS, were still inhibitory, indicating that neither boiling or freezing destroyed the anti-angiogenic activity of the RUS extract. RUS-C contained large molecules such as proteins, complex carbohydrates etc., indicating the likely active compounds could be macromolecules rather than small molecules. Conversely (or complementarily), RUS-D contained smaller molecules such as phenolic acids. RUS-D's partial inhibitory activity may indicate the presence of active compounds but in a reduced concentration due to the formation of salts after the addition of TCA as described in Example 2. RUS-E's low inhibitory activity indicates that potent inhibitors are likely to be polar compounds. The strong inhibitory activity by RUS-F indicates that potent inhibitors are likely to be polar compounds. Similar results were seen in the angiogenic index results, both with and without the zero data points. Figs. 3b and 3c.

[0067] These results indicated that fractions RUS-C and RUS-F retained potent anti-angiogenic activity. The active angiogenic inhibitors should be present in these two fractions in significant amounts that could exert a total inhibition.

Subfractions of RUS-F

[0068] The RUS-F extract was further fractionated as described in Example 2 to further isolate the compounds with anti-angiogenic activity. These extracts were then used in the human placental vein disc assay described in Example 1. PVDs were grown in HPVAM for six days. Then the HPVAM medium was supplemented with 0.1% (w/v) of one of the five fractions: RUS-F01, RUS-F02, RUS-F03, RUS-F04, and RUS-F11. The control medium was supplemented with NaCl. Twenty PVDs were used for each group. After addition of the various extracts, every two or three days, the medium in each well was replaced, and each well was scored for both initiation of angiogenesis and angiogenic index.

[0069] As shown in Fig. 4a, the initiation of angiogenesis was totally inhibited by RUS-F11, nearly completely inhibited by RUS-F03, somewhat inhibited by RUS-F01 and RUS-F04, but not inhibited

by RUS-F02. Similar results were seen in the angiogenic index both with (Fig. 4b) and without zero data points (Fig. 4c).

[0070] These results indicate that at least two active fractions of RUS-F that inhibit angiogenesis: RUS-F03 and RUS-F11.

Example 7

Inhibitory Effects of a Purified Chinese blackberry Extract (RUSF260) on Angiogenesis

[0071] To test the effectiveness of purified Chinese blackberry extracts on angiogenesis, human placental vein discs (PVD) were grown in HPVAM supplemented with a purified PLF260 fraction (0.025%). The control medium was supplemented with similar concentrations of NaCl. Two groups each with 30 PVDs were used for the control and for the PLF260 fraction. The PVDs were allowed to grow for six days in only HPVAM before adding the Chinese blackberry extract. After addition of the extract, every two to three days, the medium in each well was replaced, and each well was scored for both initiation of angiogenesis and angiogenic index.

[0072] As shown in Fig. 5a, the initiation of angiogenesis was totally inhibited by 0.025% RUSF260. Similar results were seen in the angiogenic index with and without the zero data points. See Fig. 5b and Fig. 5c. (In Figs. 5a and 5b, each data point represents an average of 30 observations; and in Fig. 5c, each data point represents an average of 20 observations.) These results indicated that the component labeled RUSF260 was a very effective anti-angiogenic agent. This component was then identified as gallic acid by the methods described in Example 2.

Example 8

Inhibitory Effects of Various Concentrations of Gallic Acid on Angiogenesis

[0073] To test the effectiveness of gallic acid on angiogenesis, gallic acid was purchased as described in Example 2, and dissolved in Medium 199 (Gibco) to reach concentrations ranging from 10^{-3} M to 10^{-10} M. These concentrations were added to PVDs that had been grown for four days in

HPVAM. After addition of the various extracts, every two to three days, the medium in each well was replaced, and each well was scored for both initiation of angiogenesis and angiogenic index.

[0074] As shown in Fig. 6a, gallic acid at 10^{-3} M totally inhibited the initiation of angiogenesis. The percent inhibition by gallic acid was dependent on the concentration. Percent initiation decreased to 56% with 10^{-4} M, 50% at 10^{-5} M. and 33% up to 10^{-10} M. Thus even at the lower concentrations, gallic acid is an inhibitor of angiogenic initiation. (In Fig. 6a, each data point represents an average of 18 to 20 observations.)

[0075] When initiation and proliferation are considered together, as measured by the mean AI with the zero points included (Fig. 6b), gallic acid at 10^{-3} M again totally inhibited angiogenesis. (In Fig. 6b, each data point represents an average of 18 to 20 observations.) However, the inhibitory effect of gallic acid stopped at concentrations of 10^{-6} M and lower. When only proliferation is considered by looking at the mean AI without the zero points (Fig. 6c), only concentrations greater than 10^{-4} M showed inhibition. (In Fig. 6c, each data point represents an average of between 5 and 11 observations.)

[0076] Thus gallic acid in concentrations greater than 10^{-4} M will be effective in inhibiting angiogenesis, with the effect being greater at 10^{-3} M.

Example 9

Inhibitory Effects of Extracts from Plants Known to Have Gallic Acid

[0077] Extracts from rhubarb, persimmon and dogwood berry were isolated as described in Example 4. These three plants were known or were found (See Example 16) to contain gallic acid. The extracts were used in the angiogenesis assay to test for inhibition of angiogenesis. PVDs were grown and prepared as described in Example 1 for eight days before addition of the extracts. The extracts were added to HPVAM to a final concentration of 0.1%. After addition of the various extracts, every two to three days, the medium in each well was replaced, and each well was scored for both initiation of angiogenesis and angiogenic index. In addition, a positive control group was established using a heparin-steroid (21-phosphate hydrocortisone) mixture (300 μg/ml and 350 μg/ml, respectively), which was previously found to reduce angiogenesis by 30 to 40%. An

untreated control group was also established. The PVDs were scored, and the media changed every two to three days.

[0078] As shown in Fig. 7a, all three extracts showed 80 to 100% inhibition of initiation of angiogenesis. (In Fig. 7a, each data point represents an average of 28 to 30 observations.) This indicates that extracts of plants known to have gallic acid are effective inhibitors of angiogenesis. A similar result was seen when initiation and proliferation are considered together as measured by the mean AI with the zero points added. (Fig. 7b; each data point represents an average of between 28 to 30 observations). However, when only proliferation is considered (Fig. 7c, mean AI without the zero points), while both rhubarb and dogwood berry inhibited the growth of angiogenesis, the persimmon extract did not. (In Fig. 7c, each data point represents an average of between 3 and 8 observations.) This may be due to differences in the other components in the extracts or in the concentration of gallic acid in the extracts.

Example 10

Effect of Tannic Acid, A Derivative of Gallic Acid, on Angiogenesis

[0079] Tannic acid is a conjugated form of gallic acid, a gallotannin, with a molecular weight of 1701.23 and a formula of $C_{76}H_{52}O_{46}$. Tannic acid can be hydrolyzed to monomers of gallic acid and glucose. Tannic acid was commercially purchased (Sigma Chemical Co., St. Louis, Missouri), and was tested in the human placental vein angiogenesis assay at a concentration of 0.1% in HPVAM. Included in the assay were an untreated control and a positive heparin-steroid control as described in Example 9.

[0080] As seen in Fig. 8a, 0.1% tannic acid inhibited the initiation of angiogenesis even better than the positive heparin-steroid control. A similar result is seen in Fig. 8b when both initiation and proliferation are considered. (In Figs. 8a, 8b, and 8c, each data point represents either an average of 10 (tannic acid), 60 (control), or 20 (heparin-steroid control) observations.) The vertical bars at the data points in Fig. 8b and 8c represent one standard error of the mean.

Example 11

Effect of Chinese Blackberry Extract or Gallic Acid on Psoriasis

[0081] To test the effectiveness of a gallic acid or a plant extract (Chinese blackberry or other) high in gallic acid on psoriasis, patients with psoriasis will be selected. All patients will be asked to continue using whatever therapy they have been using for the psoriasis. Each patient will be given two distinct 8 gm jars of a gel, one a gel with gallic acid (or a plant extract) (the "experimental gel") and one a control gel. The jars will not indicate which contains the gallic acid. The patients will be randomly divided on which arm (right or left) will be treated with the experimental gel, and which arm treated with the control gel. The patients will be asked to treat the affected area topically twice a day. Pre-study photographs of psoriatic lesions on both arms will be taken. Patients will be monitored and photographed weekly or bi-weekly. Patients will also be asked to rate the condition of their skin. An unbiased observer will also rate the lesions or plaques on the skin, using a 9-point grading system. The 9-point grading system is the sum of a grade of 0 to 3 for each of three categories -- erythema, scale, and elevation.

[0082] The experimental gel will be prepared by using purchased gallic acid or using plant extract that has been freeze-dried into a powder. The powder will then be compounded into a gel at a concentration of about 20% wt/vol, by initially using rosewater if necessary to mask any odor differences. Then Krisgel liquid, cellulose hydroxy propyl ether (Professional Compounding Centers of America, Houston, Texas), will be added to bring the volume to the final total, and the combination mixed until it gelled. The control will be the same rosewater/Krisgel liquid mixture without the gallic acid or plant extract.

[0083] It is believed that the arm treated with the gel with gallic acid or plant extract will show significant improvement over the control arm. This improvement should be reflected both by the patients objective scoring and by the rating from the independent observer.

[0084] In similar experiments, gallic acid or a plant extract will be tested for effectiveness of a topical administration against other skin disorders that involve angiogenesis, e.g., Kaposis sarcoma and some skin cancers.

Example 12

Treatment of Proliferative Retinopathies by Chinese Blackberry Extract or Gallic Acid

[0085] To test the effectiveness of gallic acid or an active extract of a plant known to contain gallic acid (Chinese blackberry or other), patients with symptoms of proliferative retinopathies, e.g., diabetic retinopathy, will be divided into two groups. One group will receive a placebo; and the other gallic acid or a plant extract, administered either by injection or orally in a tablet form, per os. The treatment will be administrated during prolonged periods of time after disease onset to inhibit pathological neovascularization. The degree of neovascularization will be followed using standard methods to measure vascularization in the eye. The treatment with gallic acid or with a plant extract high in gallic acid will result in a decrease in the degree of preexisting vascularization and will prevent the development of new angiogenic vessels.

Example 13

Oral Versus Injected Chinese Blackberry Extract for Angiogenesis Inhibition

[0086] An experiment was conducted to test the efficacy of the Chinese blackberry extract (RUS) to inhibit angiogenesis when given orally to a rat. Fifteen male Osborne-Mendel rats (300 g each; from a breeding colony at Pennington Biomedical Research Center, Baton Rouge, Louisiana) were used. Each rat was housed individually and fed rat chow ad libitum. The rats were randomized into five groups of three rats. Group 1 received no treatment and served as the control. Group 2 was injected intraperitoneally for three days with only 0.9% saline (w/v) (0.1 ml saline/100 g body weight) with a dose of 250 mg/day. Group 3 was injected intraperitoneally for three days with 250 mg of Chinese blackberry extract in 0.1 ml saline/100 mg body weight. For intraperitonial injection, the Chinese blackberry extract was vortexed and passed through a 0.22μ filter before suspension in sterile 0.9% saline (w/v). Group 4 was gavaged with 250 mg of Chinese blackberry extract (prepared as above) in 1 ml water daily for three days. Group 5 was gavaged with 750 mg of Chinese blackberry extract (prepared in saline as above) in ml water for three days. One to four hours after administering the last dose of the extract, the rats were sacrificed by guillotine and trunk blood was collected for the preparation of serum. The blood was then centrifuged to collect serum. The serum

was used in the HPVAM angiogenesis model at a concentration of 10%, supplemented with 10% fetal bovine serum to provide growth factors. In the control, 20% FBS was used. For each rat serum, 10 wells were used. HPVAM explants were observed daily under an inverted phase scope and graded for the percentage wells that became angiogenic, as well as for the angiogenic index as described in Example 1.

[0087] The results are shown in Fig. 9. Each point represents the mean angiogenic index for an individual rat. Serum from the rats that were injection with 250 mg/day of the RUS extract clearly inhibits angiogenesis when compared with the control. Although the serum from the rats given the RUS extract orally is not statistically different from the control, there is a suggestion of oral activity. In addition, there is a suggestion of a dose response since the line for the 750 mg/day dose lies below that for the 250 mg/day dose. It is believed that a larger oral dose would prove to be more effective.

Example 14

Efficacy of Chinese Blackberry Extract (RUS) on Tumors

[0088] To further characterize the effect of Chinese blackberry extract as an anti-tumor agent, two Lewis rats (Charles River Laboratories; Wilmington, Massachusetts) were implanted with CA 20948 rat pancreatic tumors (Erasmus University; Rotterdam, The Netherlands), and the tumors allowed to grow until palpable. The tumor was measured weekly by the product of the two largest diameters (tumor area cm^2). One rat was injected intraperitoneally once a week with 1 ml sterile 0.9% saline. The other rat was injected intraperitoneally once a week with 200 mg Chinese blackberry extract (0.1%) in 1 ml 0.9% saline. The extract was initially sterilized by filtering through a 0.22 micron filter.

[0089] The results are shown in Fig. 10. Fig. 10 indicates the effect of the water-soluble Chinese blackberry extract on the absolute tumor area. As seen, the weekly treatment of a tumor-bearing rat with Chinese blackberry extract effectively blocked tumor growth for 42 days (only increased 15% over baseline), while the control tumor progressively increased in size (212% over baseline).

[0090] These results indicate that the water-soluble extract of Chinese blackberry contains potent anti-tumor inhibitors, possibly due to an anti-angiogenic activity.

Example 15

Effect of Chinese Blackberry Extract on Corneal Neovascularization

[0091] To test the effects of the RUS extract on corneal neovascularization, male Long Evans pigmented rats were used. The rats were housed in individual cages and maintained under standard conditions. The experimental protocol was approved by the local Advisory Committee for Animal Resources. Forty eyes of 40 rats were used to study the effects of topical administration of the Chinese blackberry (RUS) extract or a placebo; only one eye of each animal was used as a treated or control eye.

[0092] RUS Chinese blackberry extract was reconstituted with deionized water to obtain a topical preparation. RUS extract (0.8 g) was added to 0.7 ml water and dissolved with repeated rounds of warming (37°C) followed by extending vortexing. The solution was pH adjusted to 7.0 with 1 N NaOH. Then the volume was adjusted to 8 ml and the solution was sterile-filtered. Dilutions were prepared (1:10) using sterile water. Three concentrations were prepared for topical administration (0.1%, 1%, and 10%). To ensure the sterility, the final product was filtered using a 22 micron filter prior to use.

[0093] To induce corneal vascularization in rats, a silver nitrate cauterization was used as described by J.M. Mahoney *et al.*, "Drug effects on the neovascularization response to silver nitrate cauterization of the rat cornea," Curr. Eye Res., vol. 4, pp. 531-535 (1998). All procedures were performed under general anesthesia induced by intraperitoneally administered ketamine hydrochloride and xylazine combination (94.7 mg/kg body weight). Also one drop of 0.5% topical proparacaine was applied to each cornea before the procedure. All corneas were cauterized by pressing the applicator stick (with a diameter of 1.8 mm) coated with 75% silver nitrate and25% potassium nitrate to the central cornea for 8 sec under the operating microscope. Excess silver nitrate was removed by rinsing the eyes with 10 ml of a balanced salt solution and then gently blotting them with tissue paper. To increase the reproducibility of the injuries, one investigator

cauterized all animals. Following cauterization, the rats were randomized to eliminate any potential bias in the degree of injury within the different groups. The rats were divided into four groups of ten each. Group 1 received 0.1% topical RUS extract; Group 2 received 1% topical RUS extract, Group 3 received 10% topical RUS extract, and Group 4 received saline. For each group, the treatment was administered two times a day for seven days. In all groups, treatment started immediately after cauterization.

[0094] After seven days, the animals in all groups were anesthetized as described above and their corneas evaluated by slit-lamp microscopy. Corneal photographs were taken with a 25x magnification using a digital camera attached to the slit-lamp microscope (Topcon SL-7E). Neovascularization in each cornea was evaluated using the technique of Mahoney *et al.* (1985). The evaluation was performed by an unbiased examiner. For each eye, the extent of the burn stimulus response was scored as 0 (no blister, not raised above corneal surface); +1 (small blister, raised slightly above the surface); +2 (medium blister, raised moderately above the surface); and +3 (large blister). The corneal surface covered with neovascular vessels was measured on the photographs as the percentage of the total area of the cornea. Image analysis was performed semi-automatically on each cornea using an image processing and analysis software program (BS200D-Image Analysis Software). The area of neovascularization was measured in terms of pixels, and its ratio to the entire corneal area was determined as the percentage of corneal neovascularization.

[0095] Only the corneas with a burn stimulus score of +1 or higher were included for the calculation of the mean burn stimulus and neovascularization scores in each group. Percent inhibition was calculated by comparing the mean percentage of neovascularization in each drug treated group to that in the control group. After scoring the burn stimulus and percentage of neovascularization for all groups, the animals were sacrificed in the seventh day.

[0096] For histopathology, following sedation using intraperitoneally administered ketamine hydrochloride and xylazine combination (94.7 mg/kg body weight), enucleation was performed before the animals were euthanized. Immediately after enucleation, penetration of the globe was performed with a 27-gauge needle, 1.0 mm from the limbus at the 3 and 9 o'clock meridians to allow fixative to fill the eyes rapidly. The eyes were then prepared for histologic examination using 2%

paraformaldehyde, 3% glutaraldehyde fixative. After fixation for 24 hr, the eyes were removed from the fixative, and the corneas were dehydrated and sectioned. For pre-infiltration, ethanol and Technovit 7100 were used. The eyes were infiltrated overnight using Technovit 7100. The tissues were embedded in methacrylate overnight and cut at 3 μm intervals, then stained with 1% Toluidine blue for light microscopy.

[0097] Light microscopic examination was made of every microscopic section. Sections were evaluated by dividing the corneas into two halves through the center of the lesion and evaluated with regard to the intensity of new vessels, polymorphonucleated (PMN) leukocytes, edema and fibroblastic activity. The corneas were scored as 0 (no change), +1 (mild), +2 (moderate), and +3 (severe activity). An average histopathologic score for each cornea was calculated.

[0098] Statistical analyses were performed with a one-way analysis of variance (ANOVA) test and a Mann-Whitney U test using a SPSS statistical package (SPSS for Windows; Chicago, Illinois). A p value of ≤ 0.05 was considered as statistically significant.

[0099] In all eyes, the burn stimulus score was +1 or higher. The mean burn stimulus score did not show statistically significant difference between the treatment and the placebo groups ($p=0.714$). In gross examination, all eyes treated with Chinese blackberry RUS extract showed less inflammation during the treatment period with less eyelid edema and less ciliary injection. Table 1 shows the average burn stimulus, percentage of neovascularization, histopathologic scores and inhibition percentage of test drugs on the neovascularization response in comparison with the control for each group. Topical application of 10% RUS solution caused a significant decrease in percentage of neovascularization in response to silver nitrate cauterization ($p=0.024$). Topical application of the 0.1% and 1% RUS extracts showed no significant difference compared to the control eyes ($p=0.867$, 0.455, respectively). In drug-treated and placebo eyes, the severity of burn stimulus response was positively correlated with the extent of neovascular growth, which means the lesser the intensity of the burn stimulus, the lesser the extent of neovascularization ($p<0.05$). Also, the histopathologic evaluation of Group 3 showed significantly lesser neovascularization compared to control group ($p<0.05$). (Data not shown).

Table 1. Inhibition of neovascularization by the drugs

Drug	No. eyes	Mean burn stimulus score (±SEM)[a]	Mean neovascularization % (±SEM)	Mean histopathologic score (±SEM)	% Inhibition[b]	p value
0.1% RUS	10	2.3 ± 0.66	68.06 ± 13.6	2.50 ± 0.5	12.8	0.867
1% RUS	10	2.1 ± 0.70	60.45 ± 15.4	2.40 ± 0.5	20.4	0.455
10% RUS	10	2.3 ± 0.67	30.78 ± 10.4	1.90 ± 0.5	50.0	0.024
Control	10	2.1 ± 0.56	80.86 ± 9.1	2.90 ± 0.31		

[a]**SEM** means the standard error of the mean

[b]**% Inhibition** is calculated as (1-[Mean % neovascularization for each test compound / Mean % neovascularization for the control group]) x 100

Example 16

Determination of Gallic Acid in Two* Rubus *Species and Persimmon

[0100] Leaves and berries of *Rubus fruticosus* (blackberry; obtained commercially in Baton Rouge, Louisana) were extracted separately for the analyses of gallic acid. Leaves were first oven-dried at 60°C for 72 hours and then ground to 6-mm or smaller particles. Fifty-five grams of the leaf particles were soaked for 1 hr with 500 ml deionized water (1:10 w/v) in a one-liter flask. The soaked solution was heated on a heating mantle to a boil for 30 min. The aqueous extract was centrifuged at 12,000 rpm. The supernatant was collected, concentrated in a rotary evaporator before being freeze-dried to an extract powder labeled as "RUF-L."

[0101] The cut and sifted fresh 300g berries of *Rubus fruticosus* were extracted in a similar way as in the leaves described above and an extract powder was obtained and labeled as "RUF-F."

[0102] Berries of *Rubus occidentalis* (black raspberry; obtained commercially in New Orleans, Louisiana) were extracted the same way as above and a freeze-dried extract powder was obtained and labeled as "RUO-F."

[0103] Calyx and fruit peels of persimmon (*Diospyros khaki L.*) were oven-dried and ground to 6-mm or smaller particles. The ground samples were extracted using the above described method. An extract powder was obtained and labeled as "SD."

[0104] The above-obtained extracts were analyzed for their gallic acid concentrations using HPLC by the following method: A C18 column of 150 cm long with an internal diameter of 4.6 mm with particle size 5 μm was used. The mobile phase was 10% methanol and 90% water containing 0.15% (v/v) acetic acid. A diode array UV detector was used to measure UV absorption from 190nm to 440nm. UV absorption of gallic acid was measured as a wavelength of 254nm. Gallic acid was eluated at 6.8 min. The presence of gallic acid was determined by the retention time and the UV absorption spectrum from 190nm to 440nm by the diode array. A standard calibration curve for gallic acid was developed and used to quantitate the gallic acid concentrations in the plant extracts.

[0105] RUF-L contained 0.11% gallic acid; RUF-F contained 0.02% gallic acid; RUO-F contained 0.02% gallic acid; and SD contained 0.31% gallic acid.

Example 17

Angiogenesis Inhibition of* Rubus Fruticosus *(Blackberry) Leaf and Berry Extracts and Sweet Leaf Tea (*Rubus Suavissimus*) Extract

[0106] To test if other species from the Rubus genus have anti-angiogenic activity as observed in *Rubus suavissimus*, *Rubus fruticosus* (blackberry) was tested in the HPVAM assay. Human placental vein discs (PVD) were grown in HPVAM supplemented with a leaf extract (RUF-L) and a berry extract (RUF-F) of *Rubus fruticosus*, prepared as above in Example 16. Ten PVDs per group and 30 PVDS per control and the heparin-steroid group were used. The control medium was supplemented with similar concentrations of NaCl. The positive control group was treated with a heparin-steroid (21-phosphate hydrocortisone) mixture (300 μg/ml and 350 μg/ml, respectively), which was previously found to reduce angiogenesis by 30 to 40%. The PVDs were allowed to grow for five days in only HPVAM before adding the RUF-L and RUF-F extracts. After addition of the various extracts, every two to three days, the medium in each well was replaced, and each well was scored for both initiation of angiogenesis and angiogenic index.

[0107] As shown in Fig. 11a, RUS (Sweet Leaf Tea) and RUF-L (blackberry leaf) extracts showed 100% inhibition of initiation of angiogenesis during the 14 days of culture and approximately 70% inhibition by the addition of RUF-F (blackberry berry). A similar result was seen when initiation and proliferation are considered together as measured by the mean AI with the zero points added (Fig. 11b), or when only proliferation is considered (Fig. 11c).

[0108] This indicates that two of the *Rubus* species showed similar inhibitory effects on human angiogenesis. It is believed that other *Rubus* species will contain gallic acid and other yet-to-be identified active compounds and will show comparable angiogenic activity.

Miscellaneous

[0109] The term "active plant extract" is defined as an extract from a plant that contains sufficient gallic acid to either inhibit angiogenesis or to degrade existing capillary networks. The active plant extract is an extract from a plant selected from the group consisting of *Rubus* spp., *Rubus suavissimus* (Sweet leaf tea; Chinese blackberry), *Rubus occidentalis* (North American black

raspberry), *Rubus laciniatus* (European cut-leaved blackberry), *Rubus ursinus* (Pacific blackberry or dewberry), *Rubus fruticosus* (Blackberry), *Rubus idaeus* (Raspberry), *Rubus chingii* Hu, *Rubus parviflorus* (thimbleberry), *Diospyros khaki L.* (persimmon), *Abrus prccatorius L.*; *Acacia catechu (L.) Willd.*; *Ampelopsis brevipedunculata*; *Ampelopsis japonica*; *Coriaria sinica Maxim.*; *Cornus officinalis Sieb. et Zucc.*; *Cotinus coggygria Scop.* (Smokebush); *Daucus carota L. var. Sativa DC.*; *Erodium stephanianum Willd.*; *Eucalyptus robusta Sm.*; *Euonymus bungeanus Maxim.* (Winterberry Euonymus); *Euphorbia humifusa Wild.* (Wolf's milk); *Geranium pratense L.*; *Geranium wilfordii Maxim.* (Heron's Bill); *Juglans regia L.*; *Loropetalum chinensis (R. Br.) Oliv.* (Chinese fringe tree); *Lythrum salicaria L.*; *Malus* spp. (Apple); *Mangifera indica L.* (Mango); *Macrocarpium officinale Sieb. et Zucc.*; *Passiflora caerulea L.*; *Pharbitis nil (L.) Choisy*; *Phyllanthus emblica L.*; *Pistacia chinensis Bge.*; *Platycarya longipes Wu.*; *Platycarya strobilacea Sieb. et Zucc.* (Australia cheesewood); *Polygonum aviculare L.*; *Polygonum bistorta l.* (Bistort); *Psidium guajava L.*; *Quercus infectoria Oliver*; *Rheum officinale Baill.*; *Rheum palmatum L.*; *Rheum tanguticum Maxim. Ex Reg.*; *Rhus chinensis Mill.* (Chinese sumac gallnut); *Rhus potaninii Maxim.* (Sumac gallnut); *Rosa chinensis Jacq.* (Mini rose); *Rosa rugosa Thunb.* (Rose); *Rubus ulmifolius*; *Rumex japonicus Houtt.* (Japanese dock); *Sanguisorba officinalis L.* (Burnet); *Sapium sebiferum (L.) Roxb.*; *Syzygium cumini (L.) Skeels*; *Tamarix chinensis Lour.*; *Terminalia chebula Retz.* (Medicine terminalia); *Tetrastigma hypoglaucum Planch.*; and *Tussilago farfara L.*.

[0110] The term "therapeutically effective amount" as used herein refers to an amount of gallic acid or of an "active plant extract" sufficient either to inhibit angiogenesis or to degrade existing capillary networks to a statistically significant degree ($p<0.05$). The term "therapeutically effective amount" therefore includes, for example, an amount sufficient to prevent the growth of angiogenic vessels found in diseases of tumor growth, diabetic retinopathy, psoriasis, retinopathy of prematurity, rheumatoid arthritis, and preferably to reduce by at least 50%, and more preferably to reduce by at least 90%, the amount of angiogenesis. The dosage ranges for the administration of gallic acid or the active plant extract are those that produce the desired effect. Generally, the dosage will vary with the age, weight, condition, sex of the patient, type of tumor or other pathology, the degree of tumor development, and the degree of angiogenic response. A person of ordinary skill in the art, given the

teachings of the present specification, may readily determine suitable dosage ranges. The dosage can be adjusted by the individual physician in the event of any contraindications. In any event, the effectiveness of treatment can be determined by monitoring the extent of angiogenic inhibition or remission by methods well known to those in the field. Moreover, gallic acid or the active plant extract can be applied in pharmaceutically acceptable carriers known in the art. Gallic acid or the active plant extract can be used to treat cancers in animals and in humans *in vivo*. The application can be oral, by injection, or topical, providing that in an oral administration gallic acid or the active plant extract is preferably protected from digestion.

[0111] Gallic acid or the active plant extract may be administered to a patient by any suitable means, including parenteral, subcutaneous, intrapulmonary, topically, and intranasal administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal or intravitreal administration. Additionally, the infusion could be into an organ or tumor or site of disease. Injection of gallic acid or its active plant extract may include the above infusions or may include intraperitonieal, intravitreal, direct injection into a tumor, or direct injection into a site of angiogenic disease. Gallic acid or the active plant extract may also be administered transdermally, for example in the form of a slow-release subcutaneous implant, or orally in the form of capsules, powders, or granules. Although direct oral administration seems to cause loss of antiangiogenic activity, gallic acid or the active plant extract could be packaged in such a way to protect the active ingredient(s) from digestion by use of enteric coatings, capsules or other methods known in the art.

[0112] Pharmaceutically acceptable carrier preparations for parenteral administration include sterile, aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. The active therapeutic ingredient may be mixed with excipients that are pharmaceutically acceptable and are compatible with the active ingredient. Suitable excipients include water, saline, dextrose, and glycerol, or combinations thereof. Intravenous vehicles include fluid and nutrient replenishers, electrolyte

replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases, and the like.

[0113] The form may vary depending upon the route of administration. For example, compositions for injection may be provided in the form of an ampule, each containing a unit dose amount, or in the form of a container containing multiple doses.

[0114] Gallic acid or the active plant extract may be formulated into therapeutic compositions as pharmaceutically acceptable salts. These salts include the acid addition salts formed with inorganic acids such as, for example, hydrochloric or phosphoric acid, or organic acids such as acetic, oxalic, or tartaric acid, and the like. Salts also include those formed from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and organic bases such as isopropylamine, trimethylamine, histidine, procaine and the like.

[0115] Controlled delivery may be achieved by admixing the active ingredient with appropriate macromolecules, for example, polyesters, polyamino acids, polyvinyl pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, prolamine sulfate, or lactide/glycolide copolymers. The rate of release of gallic acid or the active plant extract may be controlled by altering the concentration of the macromolecule.

[0116] Controlled delivery can also be achieved by conjugating gallic acid with a known compound that targets cellular surface receptors that are known to be unique to angiogenic blood vessels, e.g., somatostatin and its analogs and derivatives (binding to somatostatin receptor subtype 2), platelet-derived growth factor (binding to platelet derived growth factor receptor), and vascular endothelial growth factor(binding to a kdr receptor). See M.O. Meyers *et al*, "Gene upregulation of PDGF in human angiogenesis," abstract presented at Association for Academic Surgery, 1998; J.C. Watson *et al.*, "SST-2 gene expression appears during human angiogenesis," abstract published in Regul. Peptide, vol. 64, pp. 206 (1996); J.C. Watson *et al.*, "Initiation of *kdr* gene transcription is associated with conversion of human vascular endothelium to an angiogenic phenotype," Surgical Forum, vol. 47, pp. 462-464 (1996); and J.C. Watson *et al.*, "Growing vascular endothelial cells express somatostatin subtype 2 receptors," British Journal of Cancer, vol. 85, pp. 266-272 (2001).

[0117] Another method for controlling the duration of action comprises incorporating gallic acid or the active plant extract into particles of a polymeric substance such as a polyester, peptide, hydrogel, polylactide/glycolide copolymer, or ethylenevinylacetate copolymers. Alternatively, gallic acid or the active plant extract may be encapsulated in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, by the use of hydroxymethylcellulose or gelatin-microcapsules or poly(methylmethacrylate) microcapsules, respectively, or in a colloid drug delivery system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

[0118] The present invention provides a method of preventing, treating, or ameliorating a disease that causes an angiogenic response in the body such as retinopathy and psoriasis, comprising administering to a subject at risk for a disease or displaying symptoms for such disease, a therapeutically effective amount of gallic acid or an active plant extract. The term "ameliorate" refers to a decrease or lessening of the symptoms or signs of the disorder being treated. The symptoms or signs that may be ameliorated include those associated with an increase in angiogenesis in the body.

[0119] The complete disclosures of all references cited in this specification are hereby incorporated by reference. In the event of an otherwise irreconcilable conflict, however, the present specification shall control.

ABSTRACT OF THE DISCLOSURE

An extract of Chinese blackberry (*Rubus suavissimus*) was found to inhibit angiogenesis. From the extract, a powerful anti-angiogenic compound was isolated and found to be gallic acid. The antiangiogenic activity was measured by an assay that is an *in vitro* human angiogenesis model using a human placental vein disc. Extracts from other plants either known or found to have gallic acid, e.g., rhubarb root, persimmon fruit, blackberry (*Rubus fruticosus*), and dogwood berry, were also found to have anti-angiogenic activity. Gallo tannin (tannic acid) was also found to inhibit angiogenesis. Other derivatives of gallic acid will be tested for their anti-angiogenic activity, including methyl gallate, ethyl gallate, propyl gallate, butyl gallate, lauryl gallate, octyl gallate, ellagic acid, BUSMUTH-gallate, galloyl glucose, di-galloyl glucose, tri-galloyl glucose, tetra-galloyl glucose, and penta-galloyl glucose. The extract from Chinese blackberry also slowed the growth of a pancreatic tumor and of corneal neovascularization in rats. Extracts from *Rubus* spp, and other plants with gallic acid, and gallic acid will be useful for treating various diseases associated with neovascularization, including diabetic retinopathy, psoriasis, tumors, obesity, cancer, rheumatoid arthritis, etc.

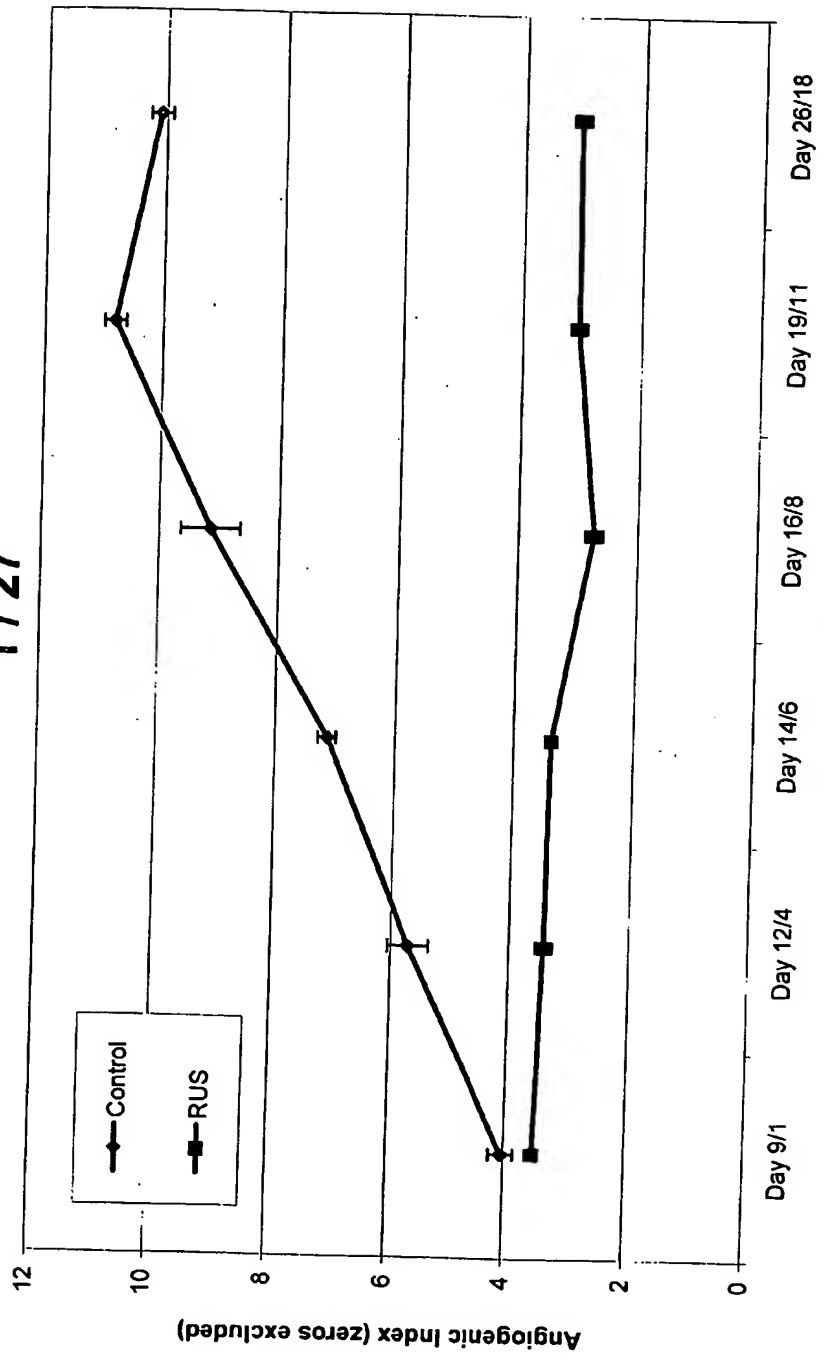

Fig. 1

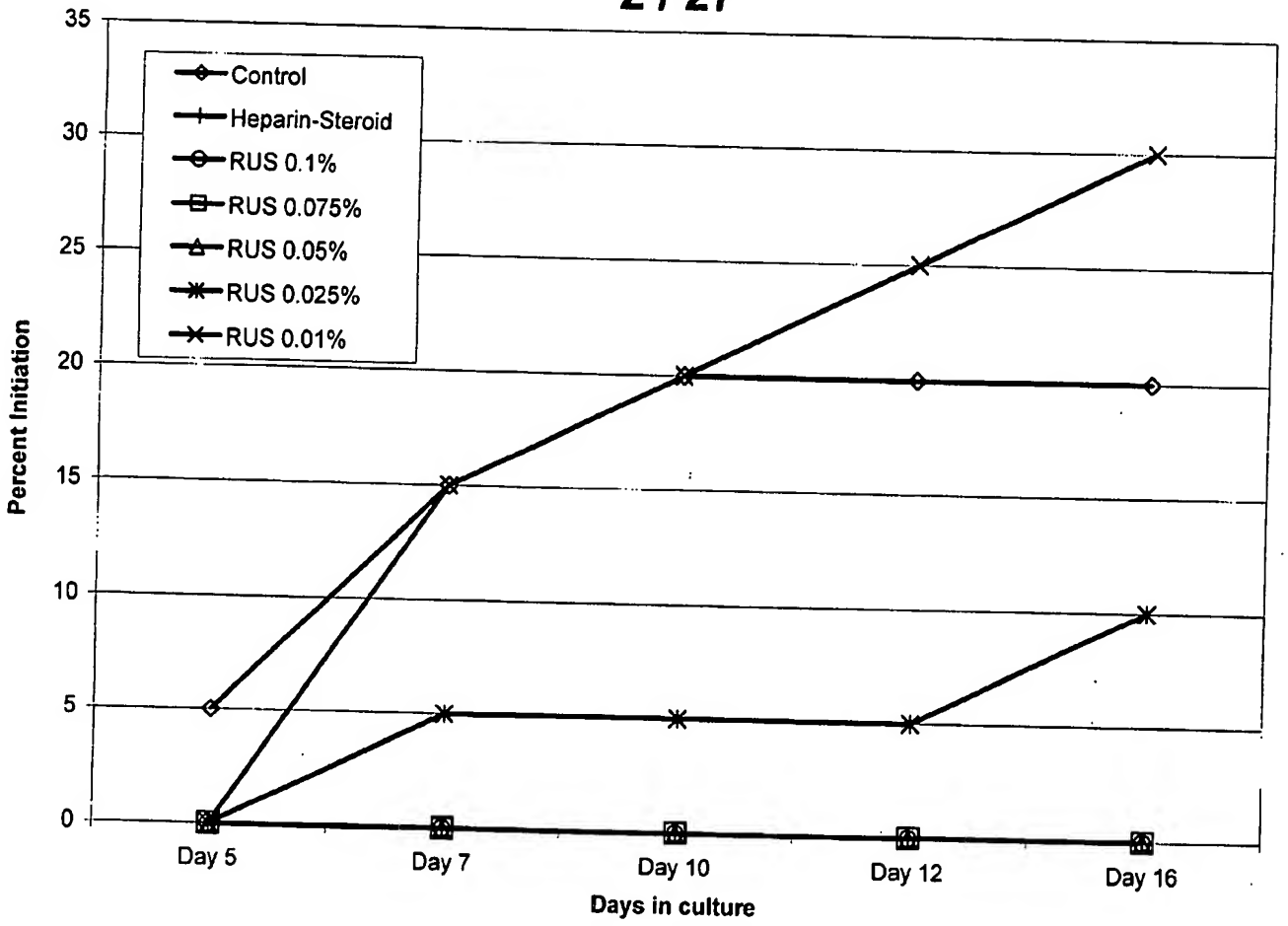

Fig. 2A

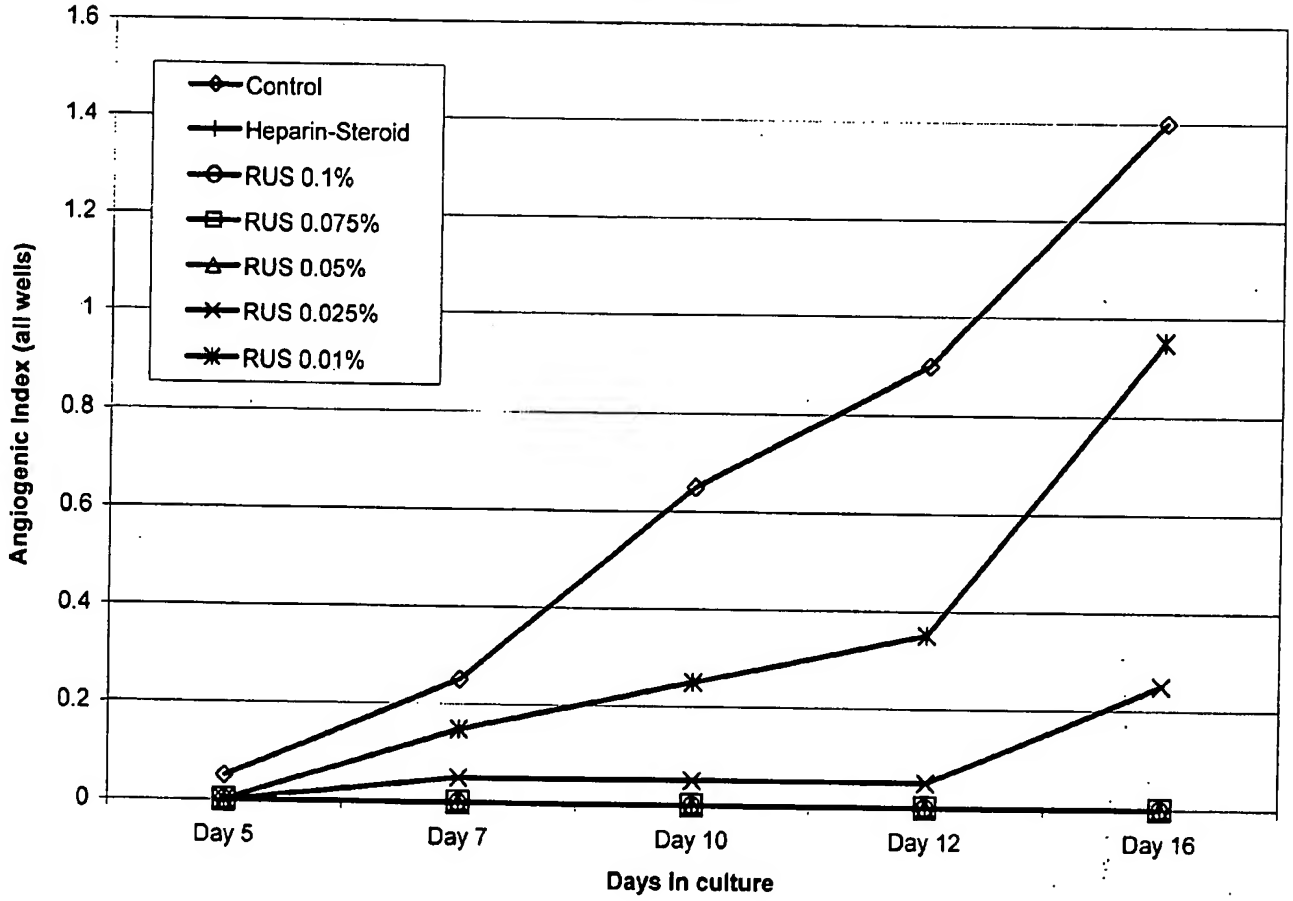

Fig. 2B

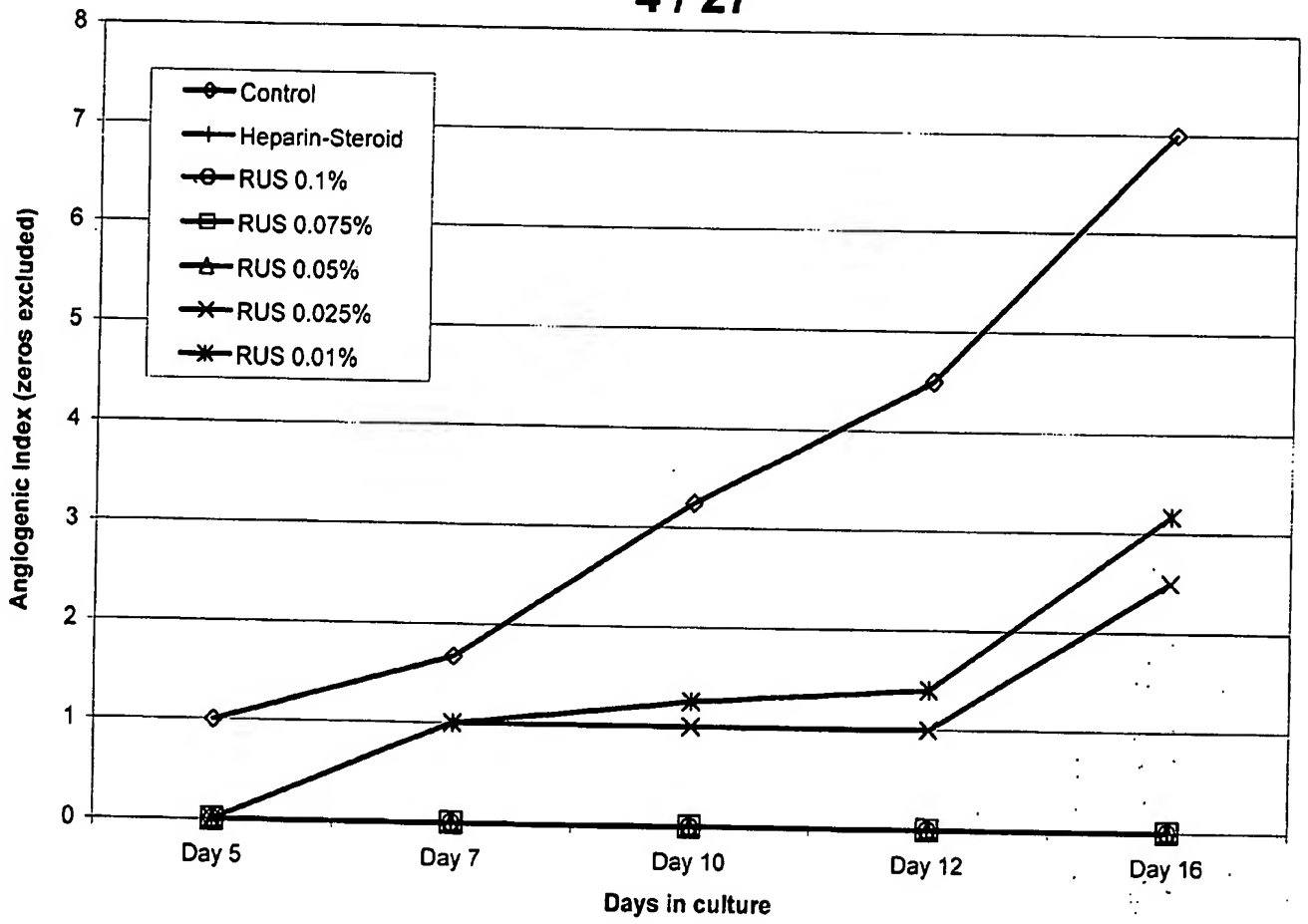

Fig. 2C

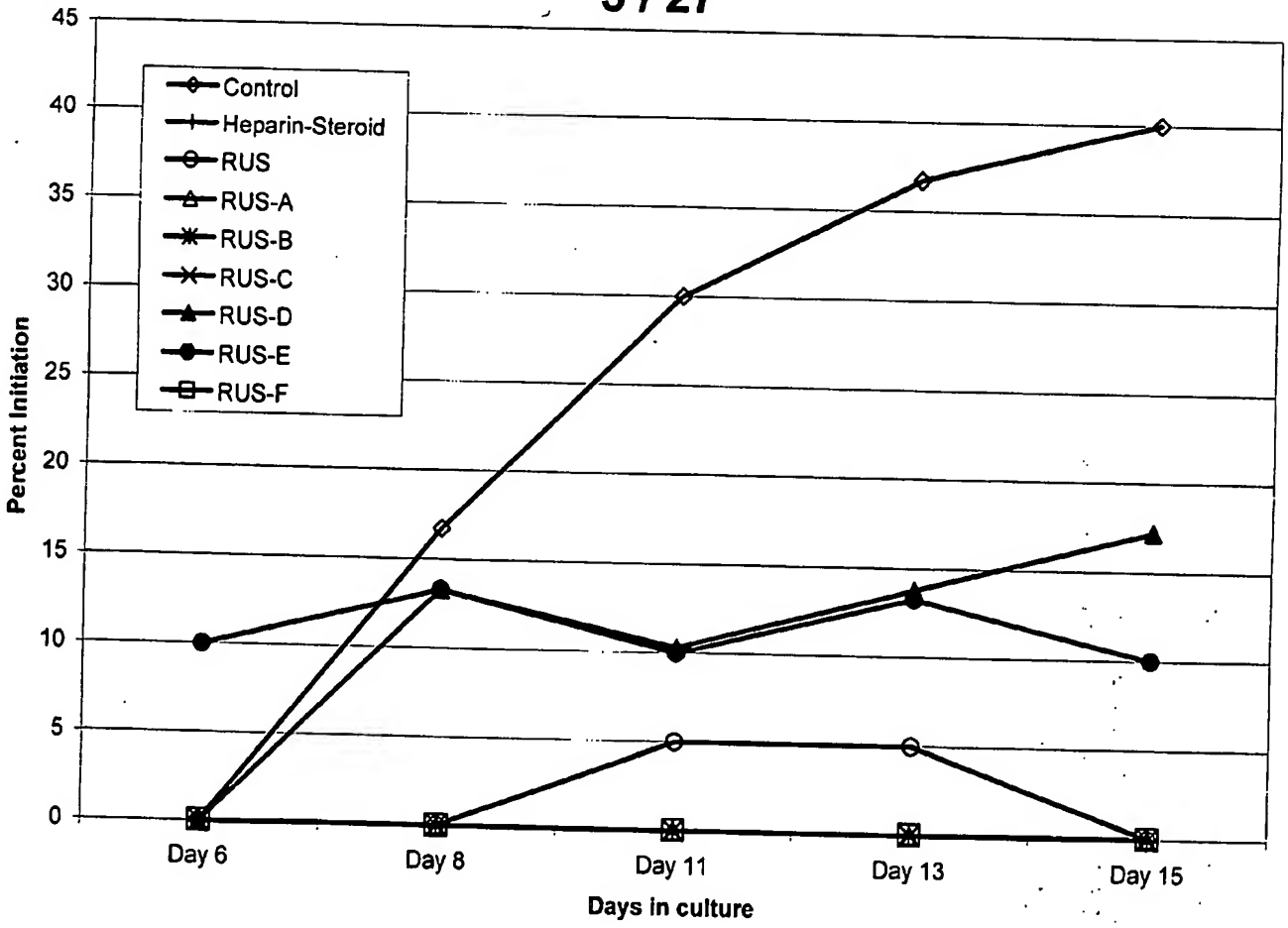

Fig. 3A

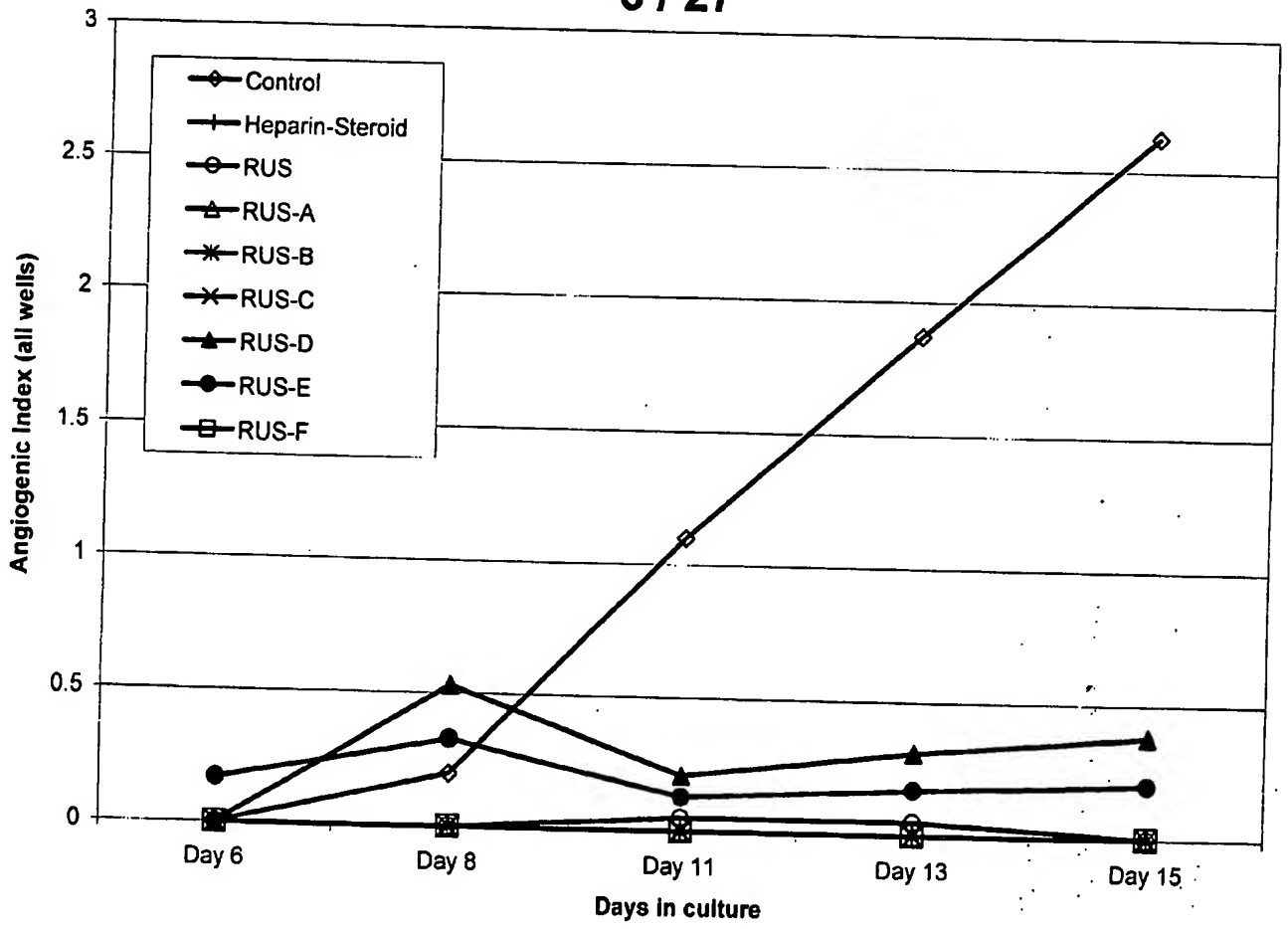

Fig. 3B

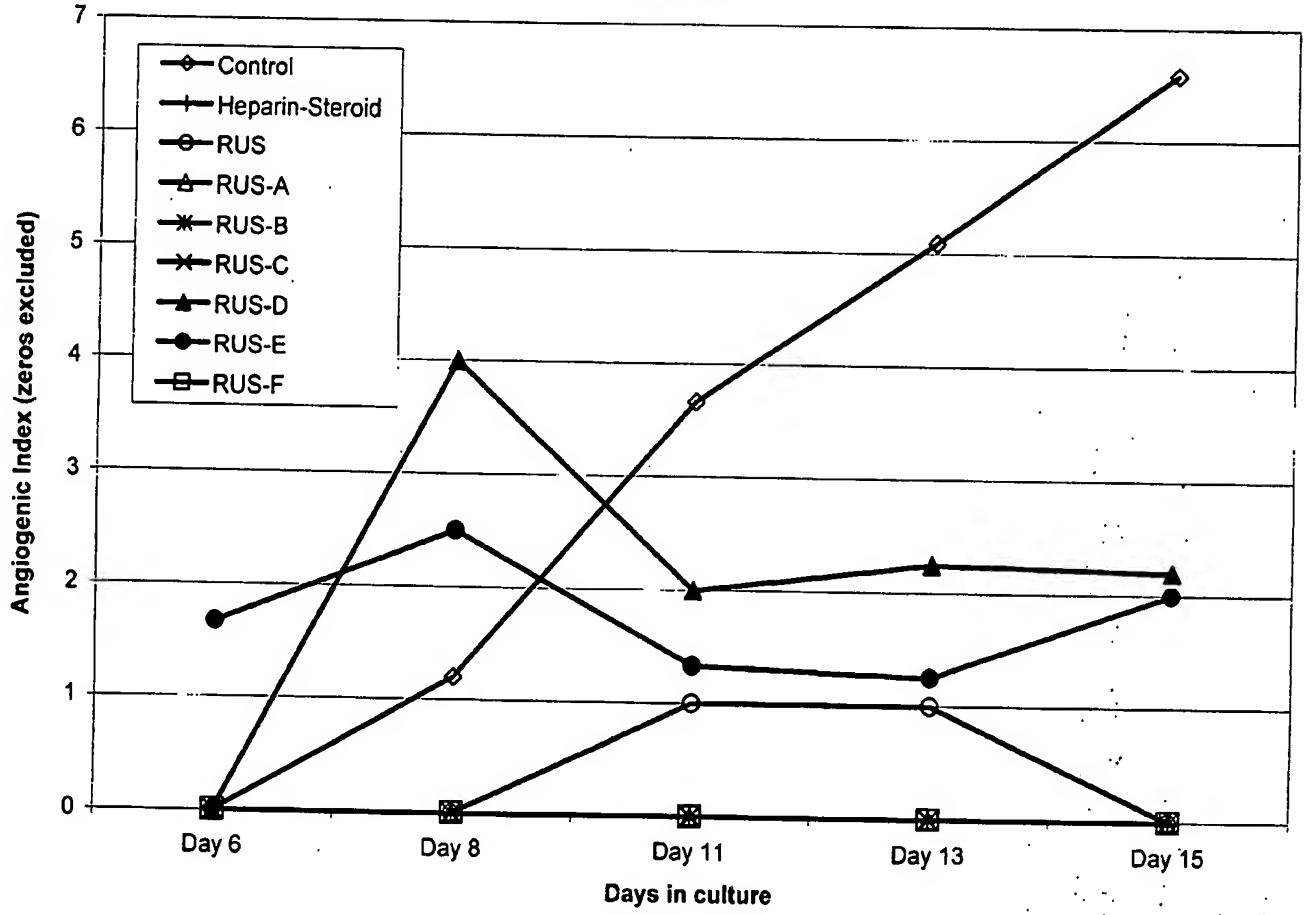

Fig. 3C

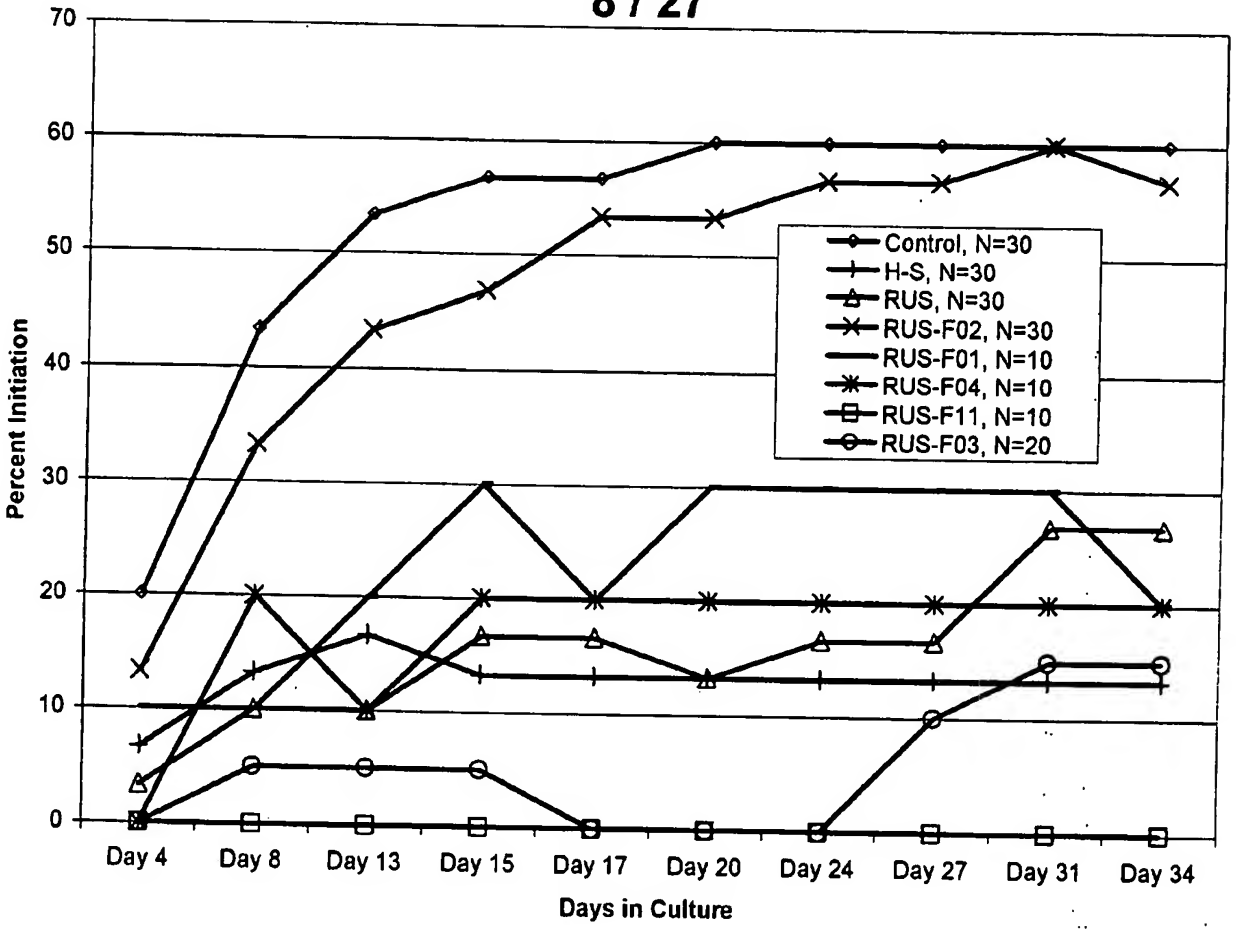

Fig. 4A

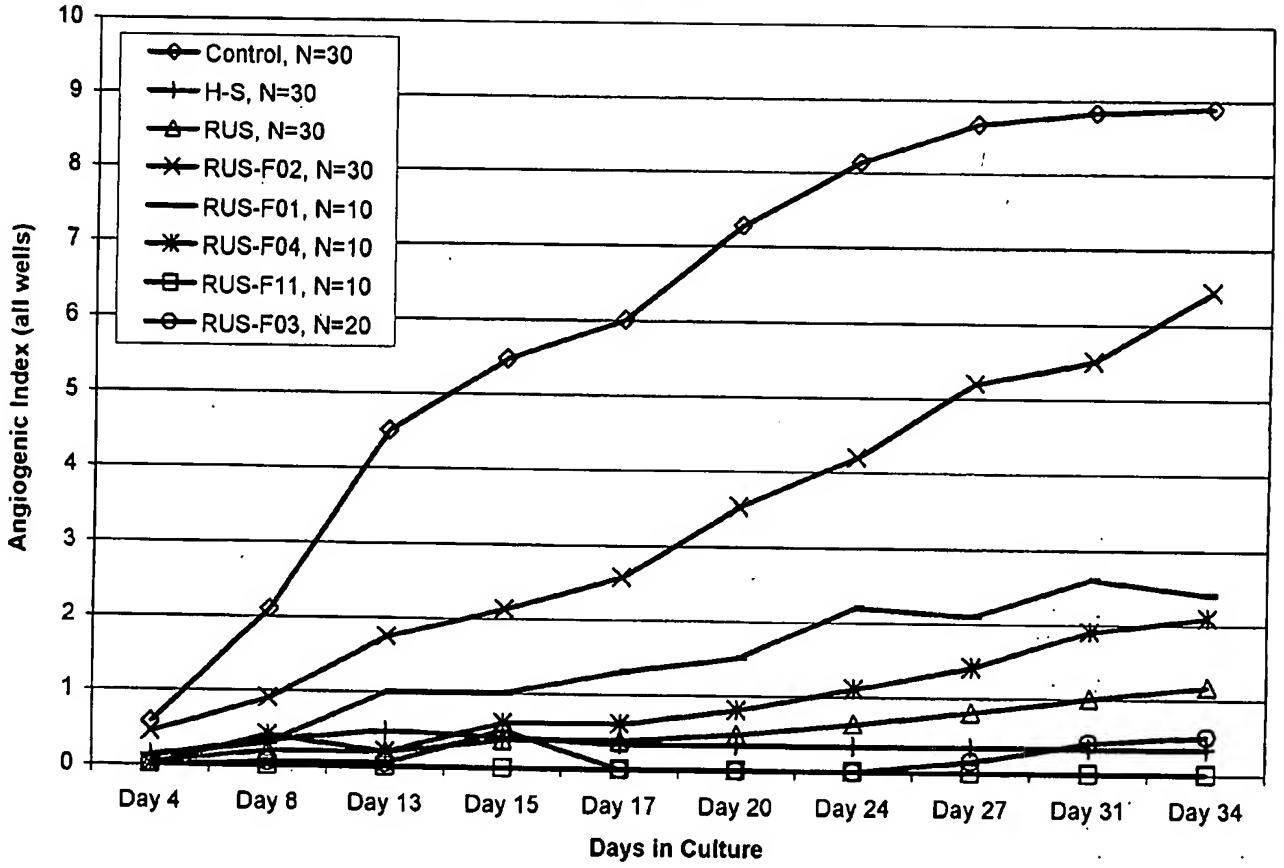

Fig. 4B

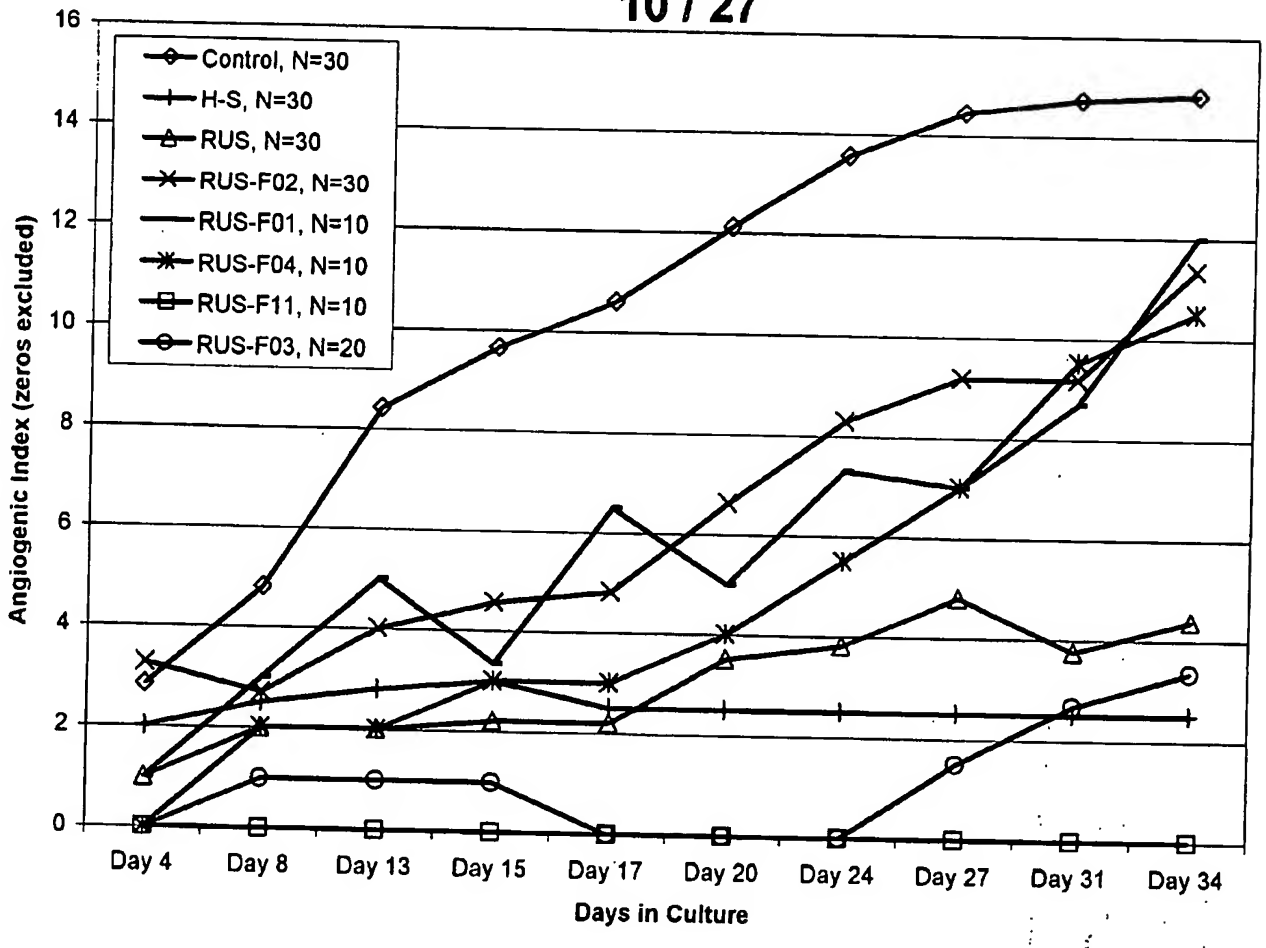

Fig. 4C

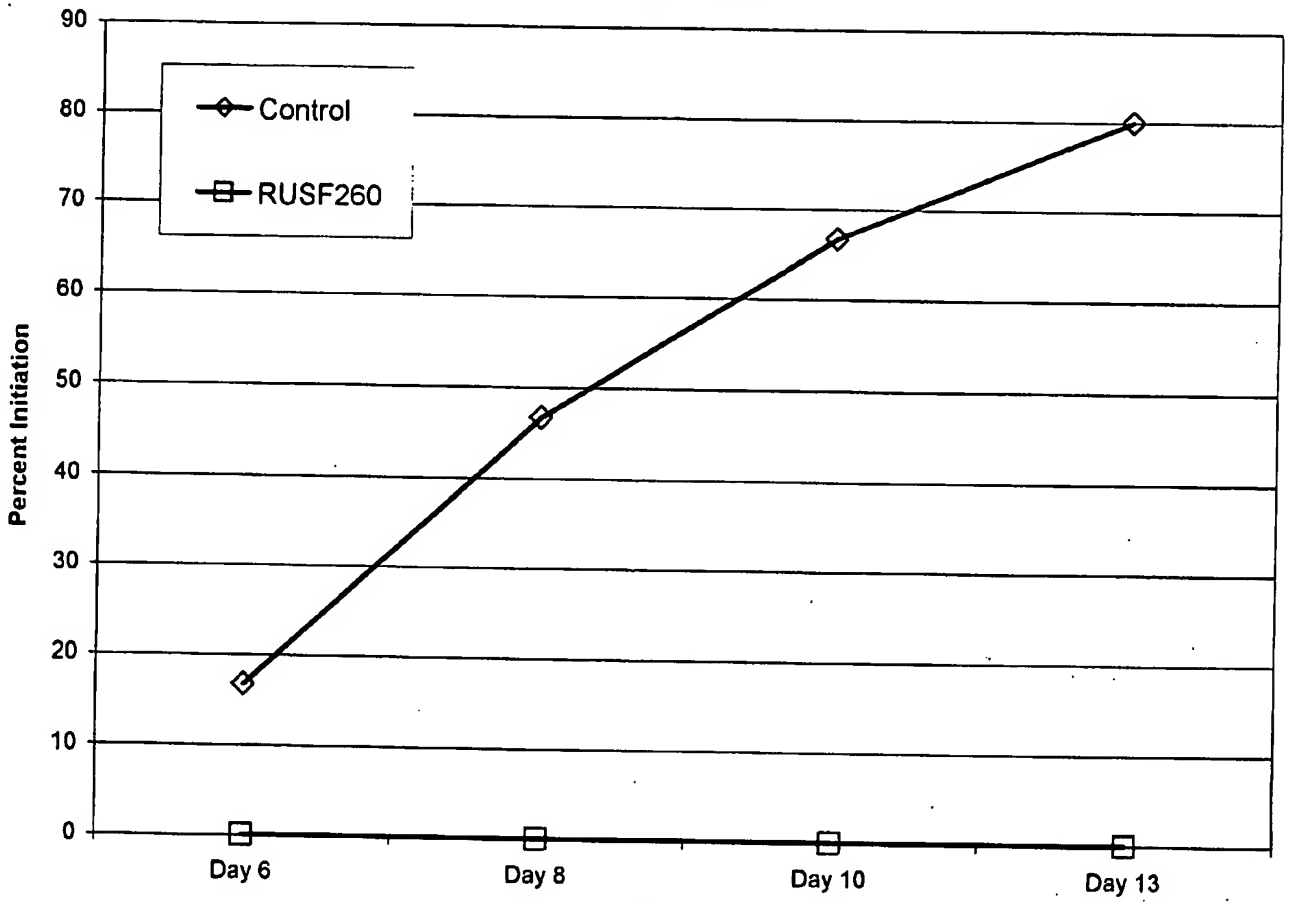

Fig. 5A

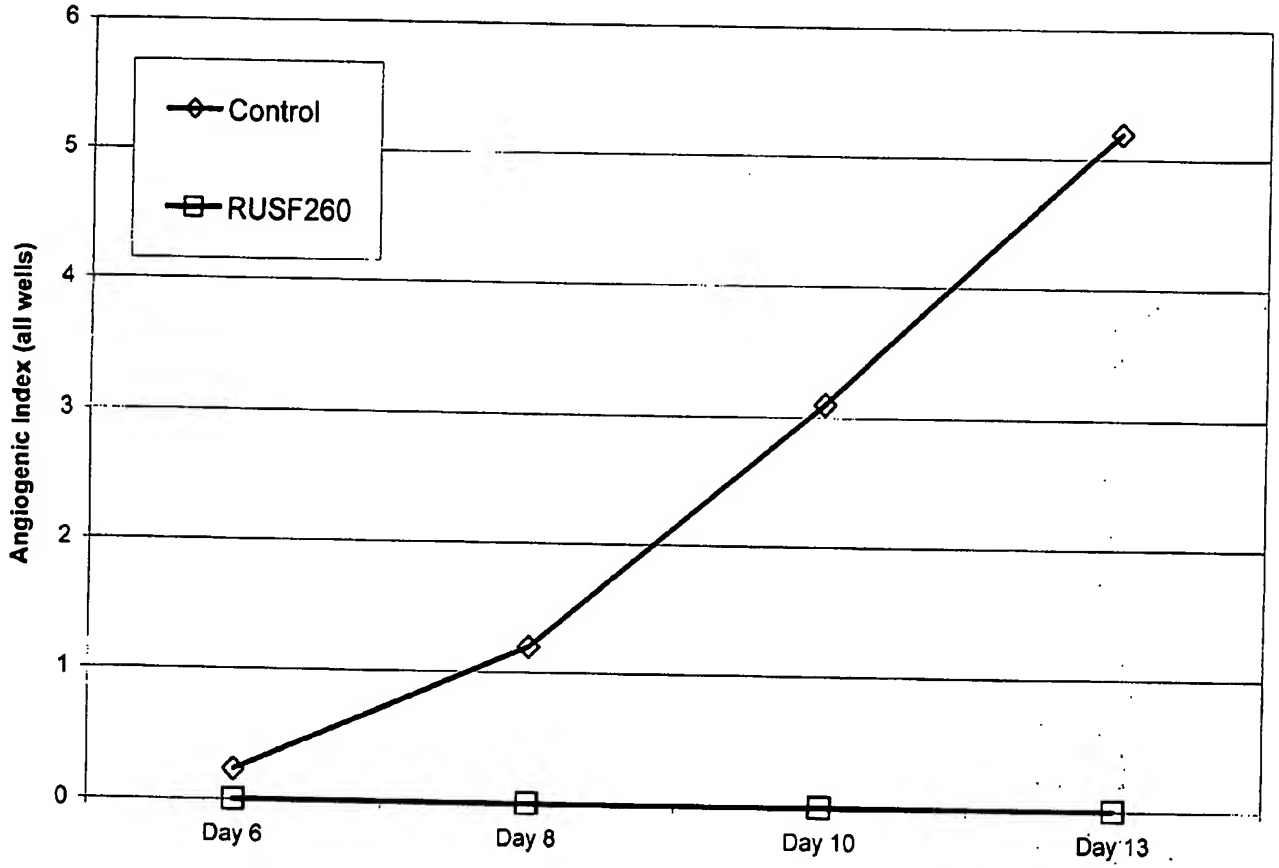

Fig. 5B

Angiogenic Index (zeros excluded)
0
1
2
3
4
5
6
7
Day 6
Day 8
Day 10
Day 13
Control
RUSF260

Fig. 5C

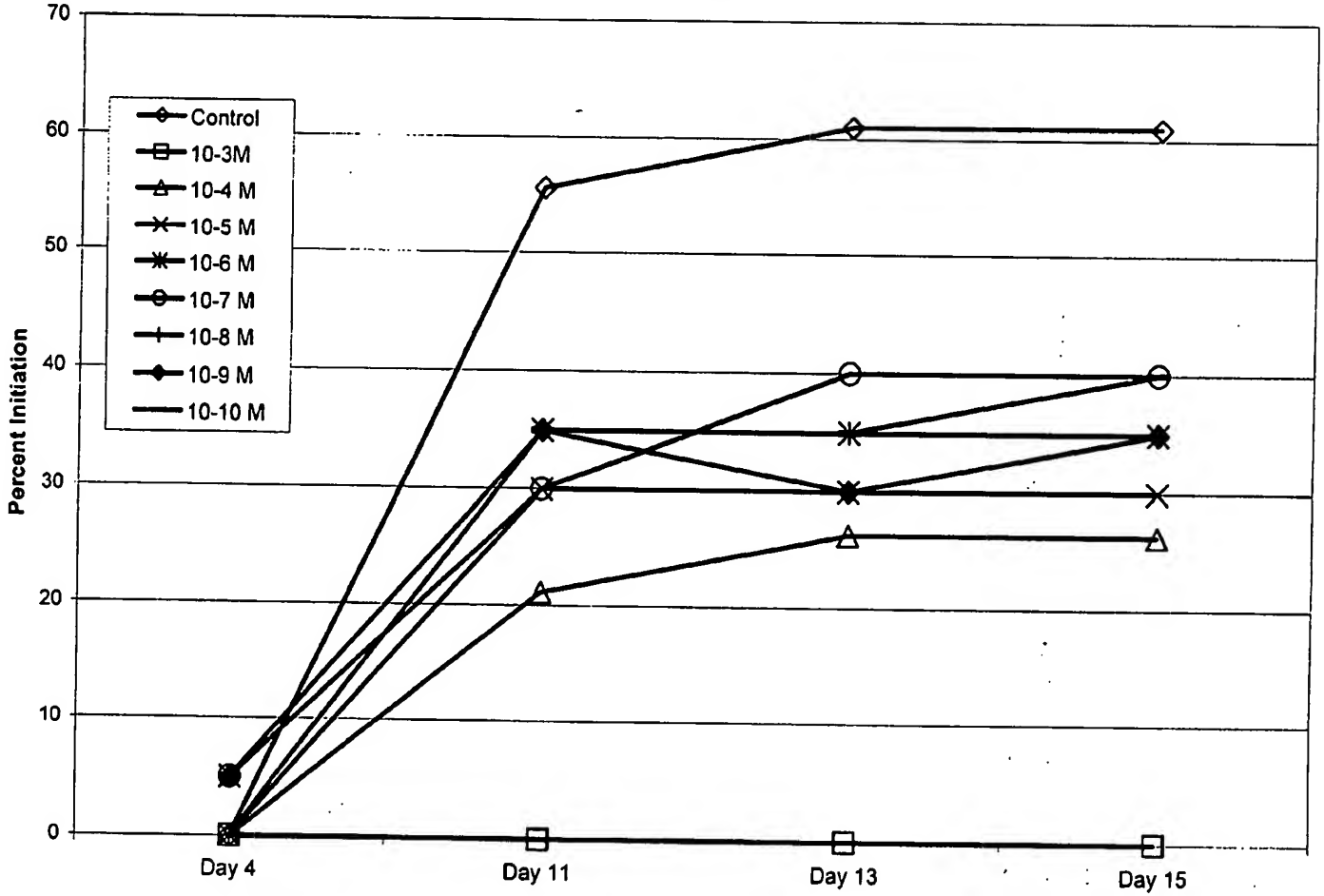

Fig. 6A

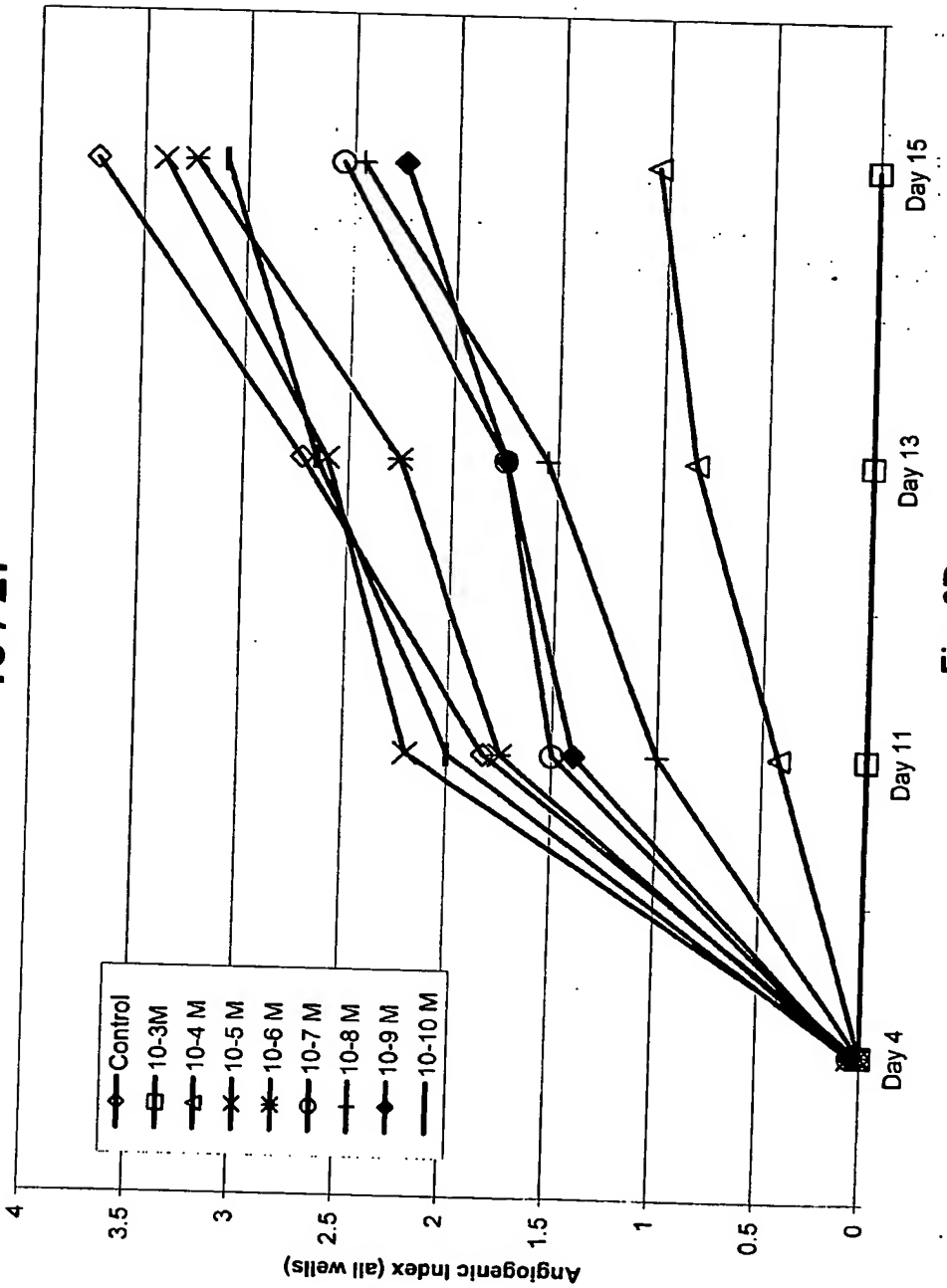

Fig. 6B

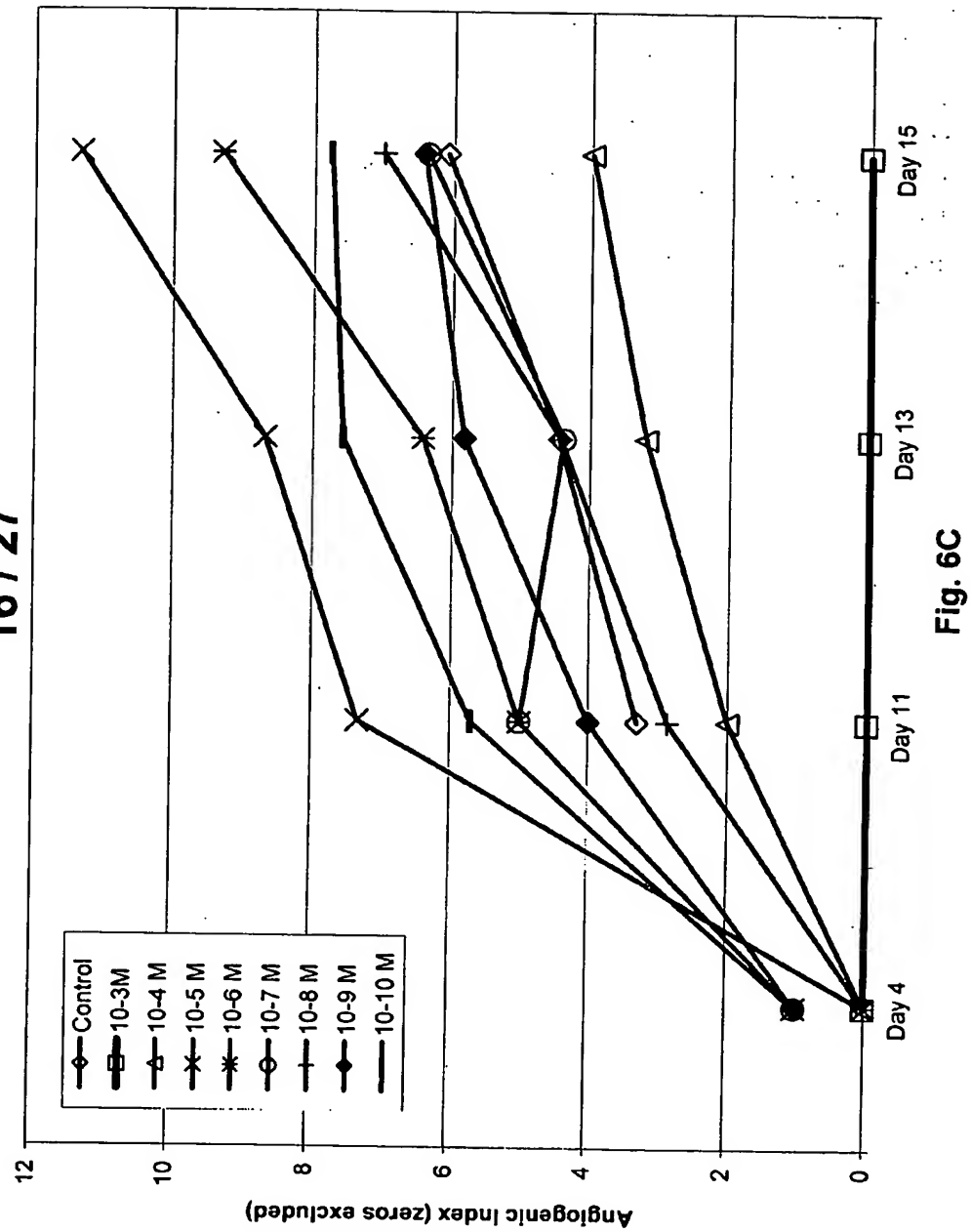

Fig. 6C

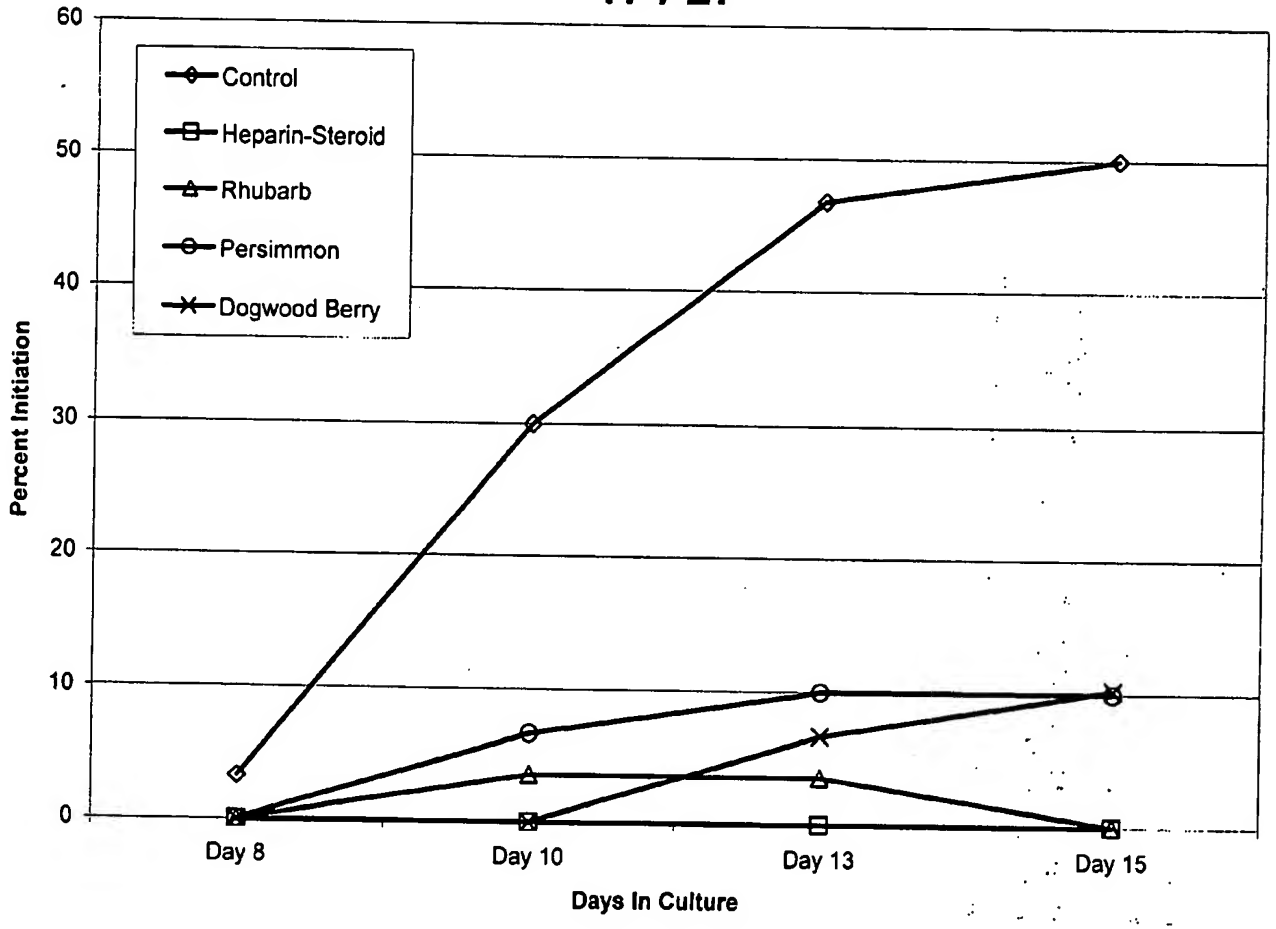

Fig. 7A

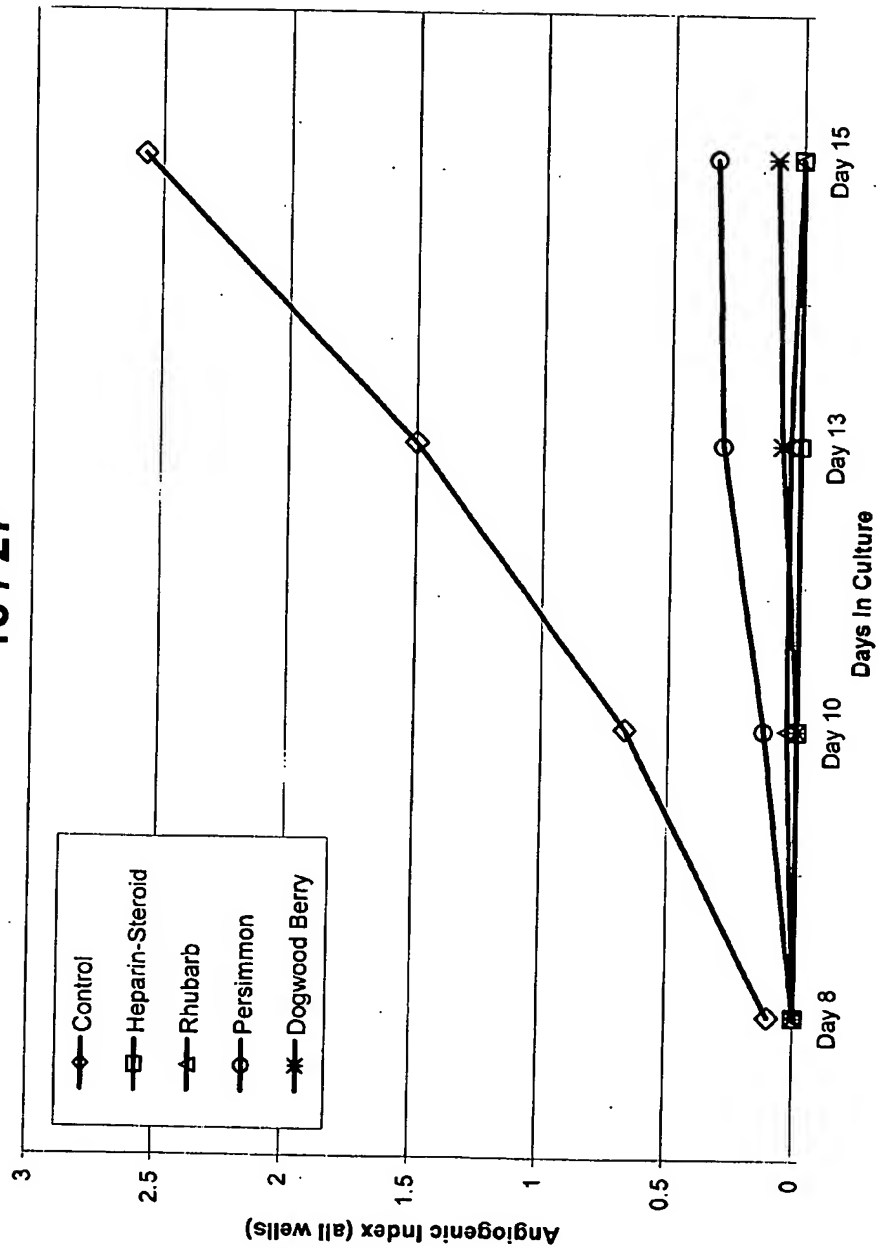

Fig. 7B

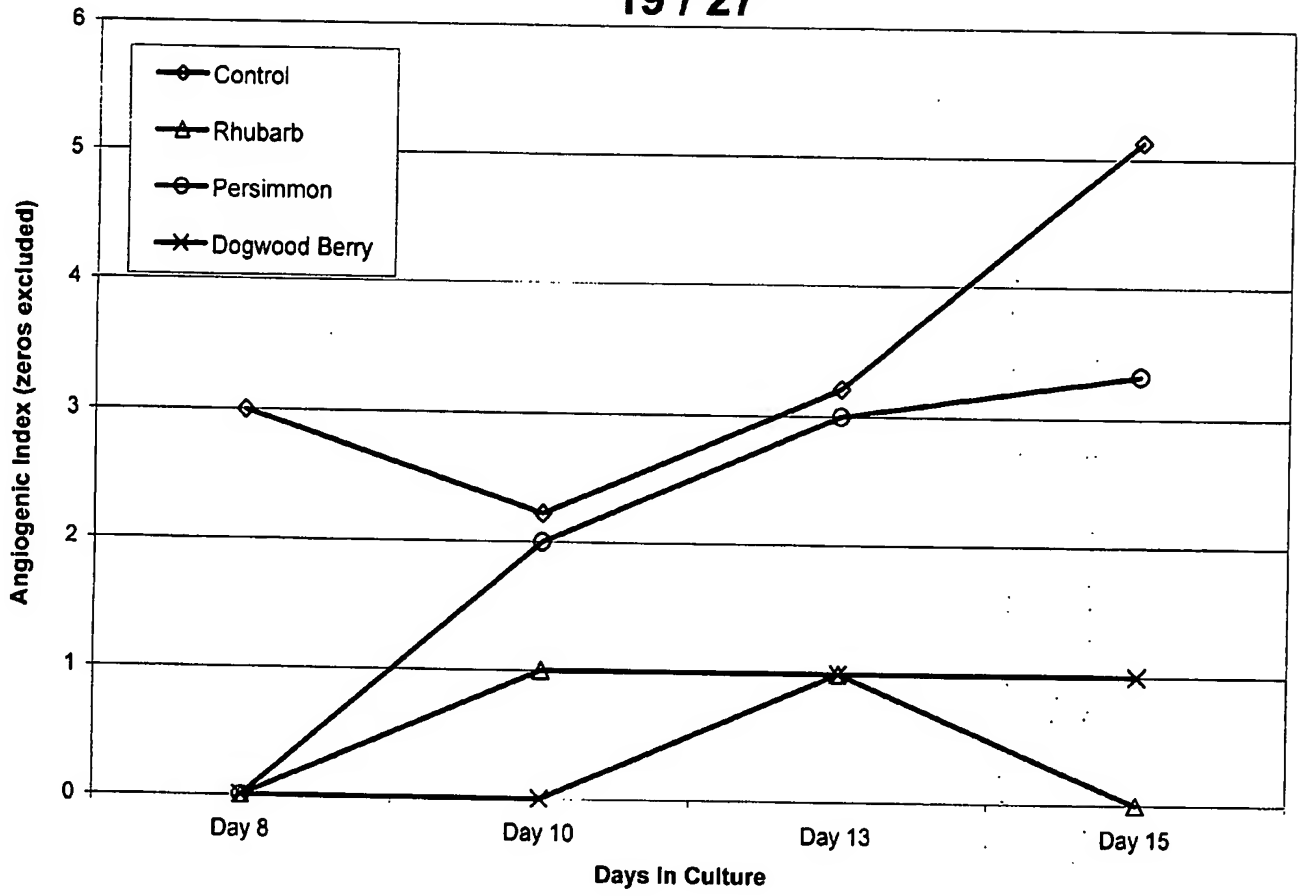

Fig. 7C

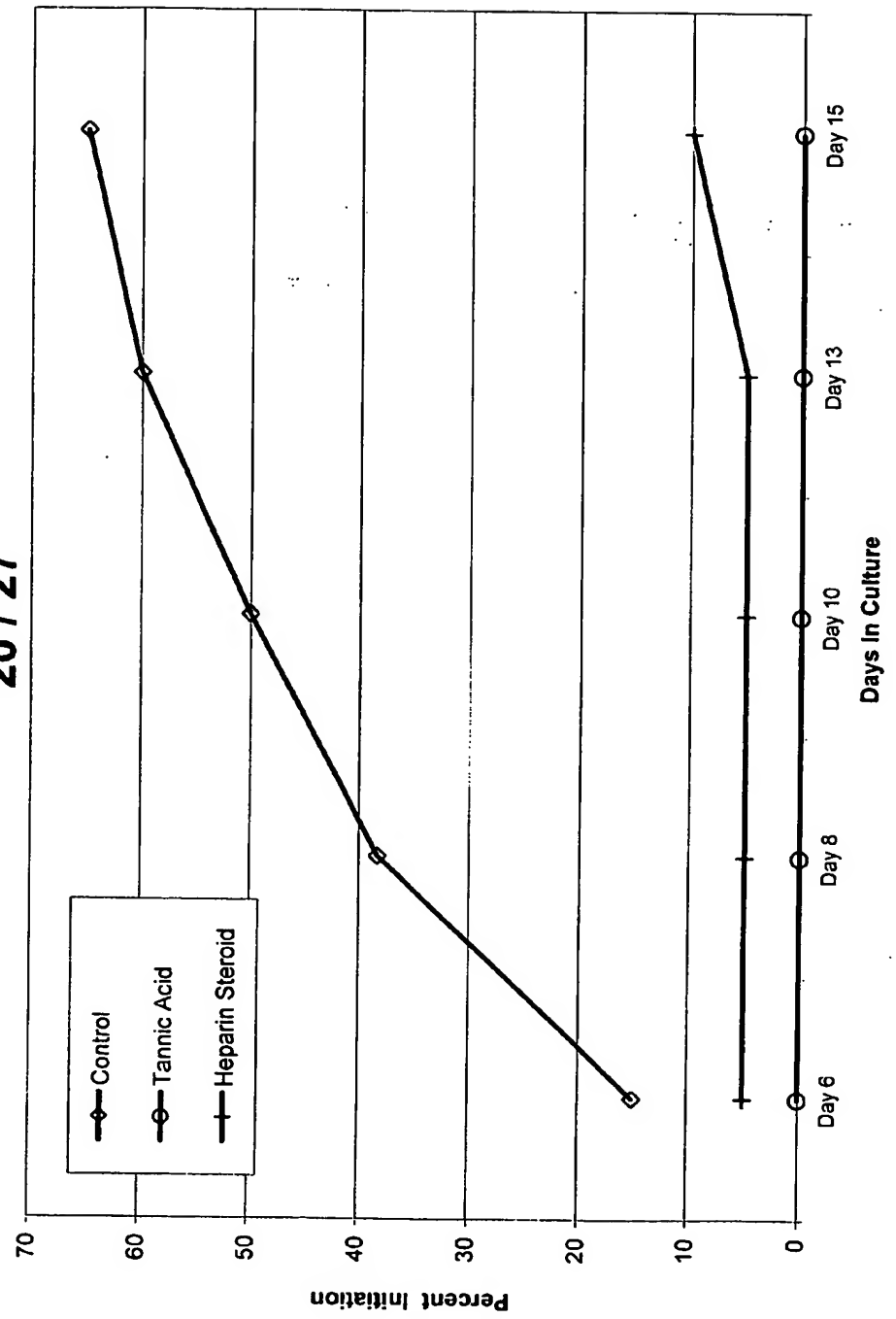
Control
Tannic Acid
Heparin Steroid
Percent Initiation
70
60
50
40
30
20
10
0
Day 6
Day 8
Day 10
Day 13
Day 15
Days In Culture

Fig. 8A

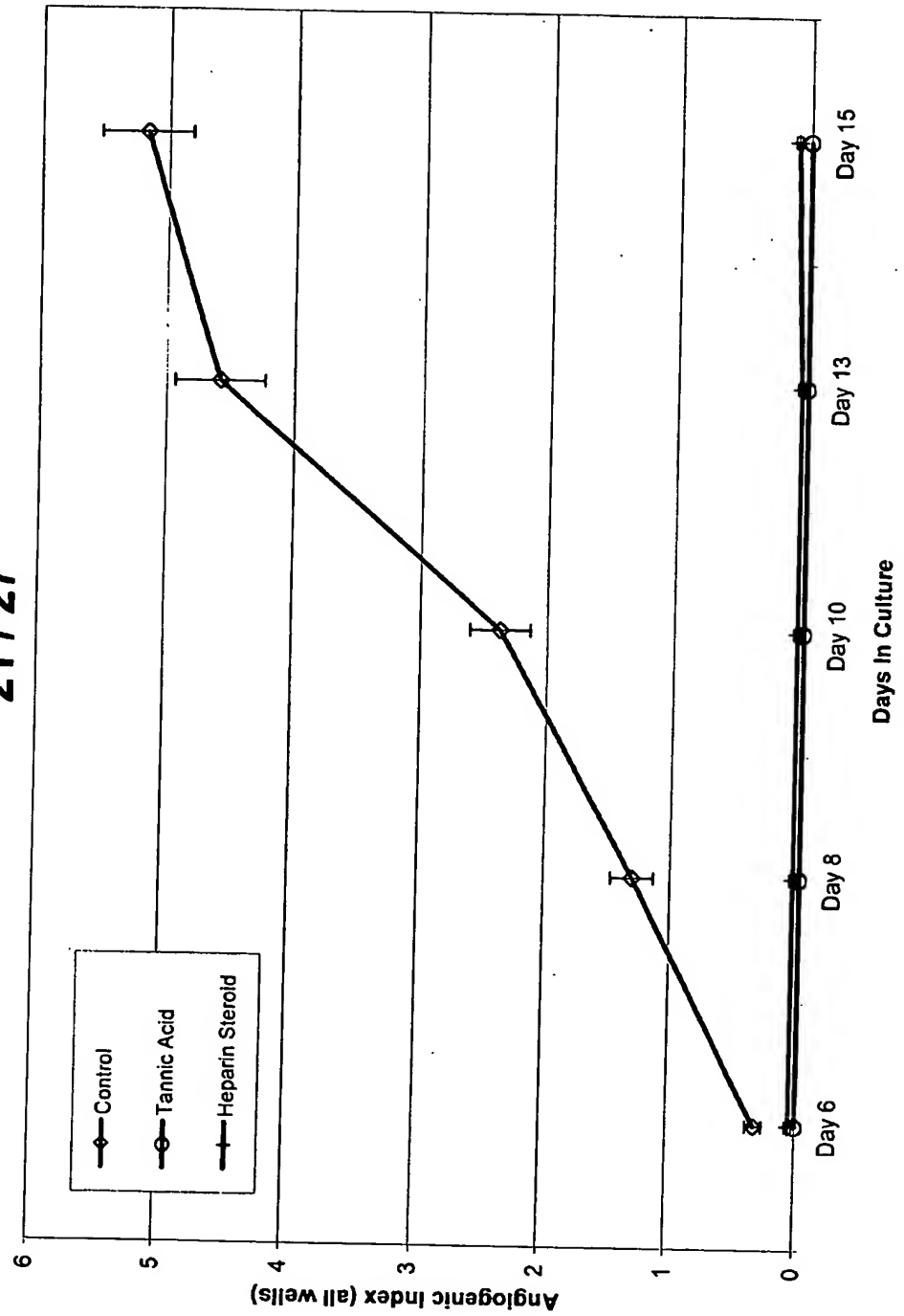
Angiogenic Index (all wells)
0
1
2
3
4
5
6
Control
Tannic Acid
Heparin Steroid
Day 6
Day 8
Day 10
Day 13
Day 15
Days In Culture

Fig. 8B

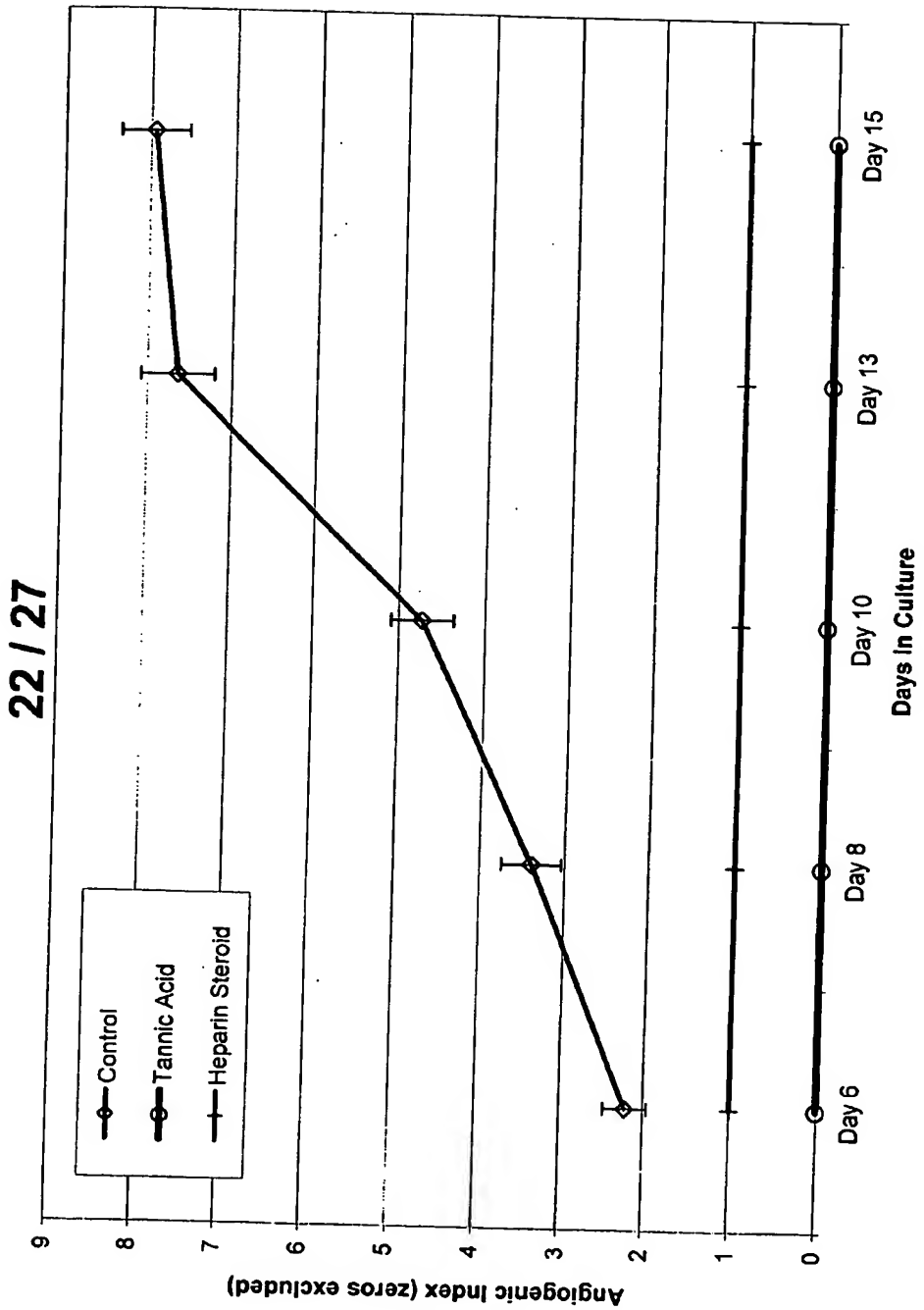
Angiogenic Index (zeros excluded)
0
1
2
3
4
5
6
7
8
9
Day 6
Day 8
Day 10
Day 13
Day 15
Days In Culture
Control
Tannic Acid
Heparin Steroid

Fig. 8C

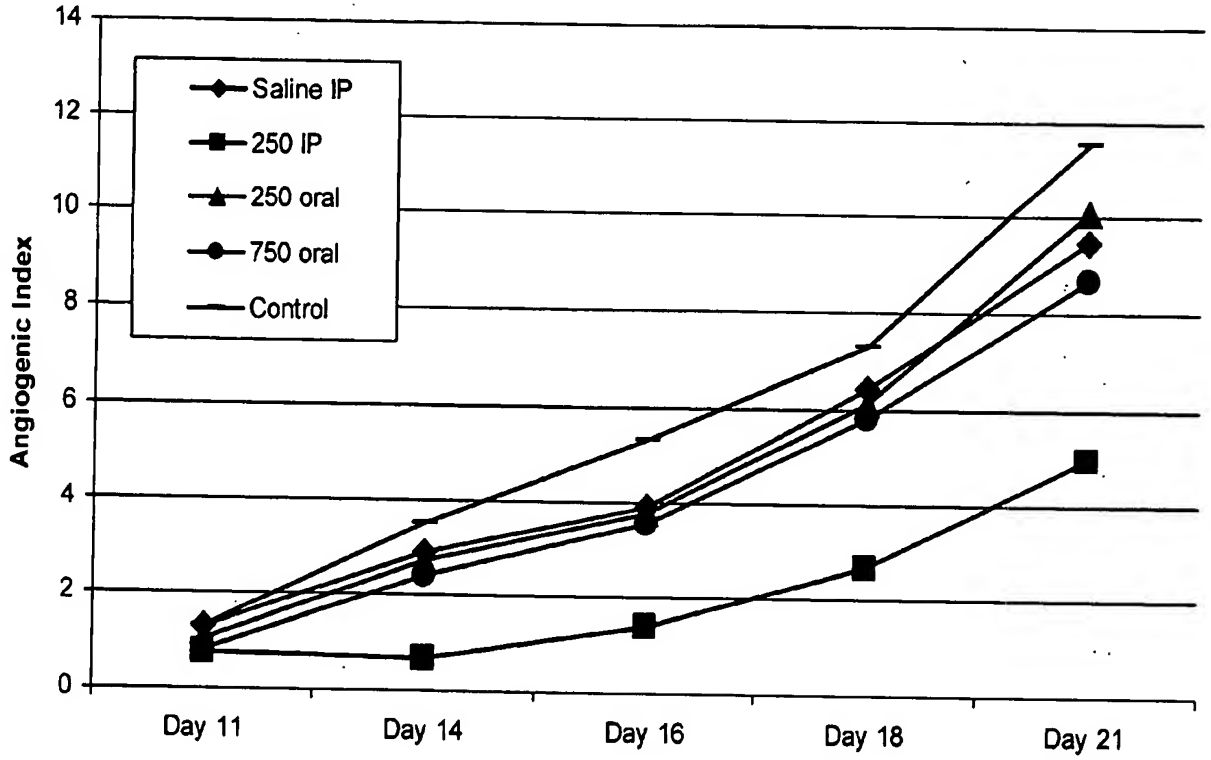

Fig. 9

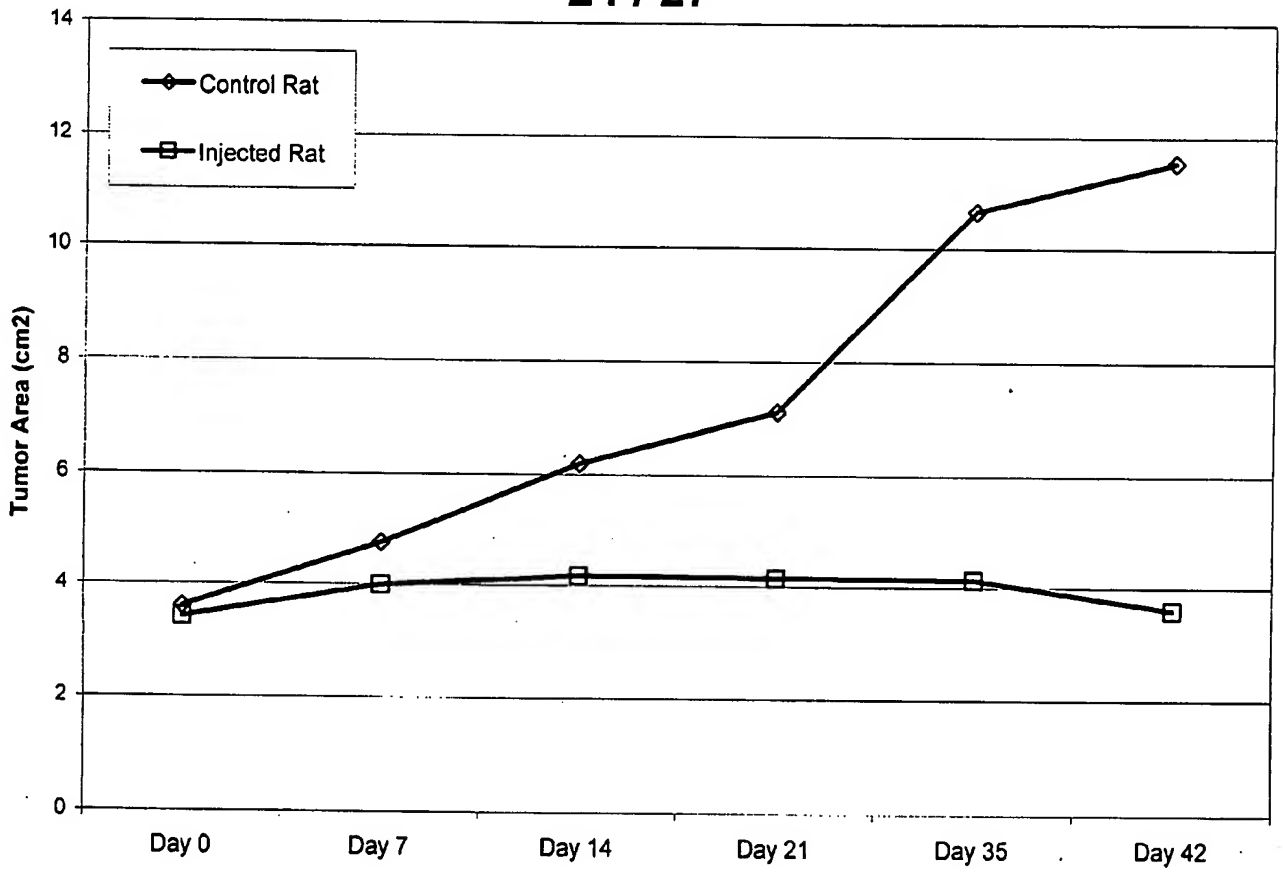

Fig. 10

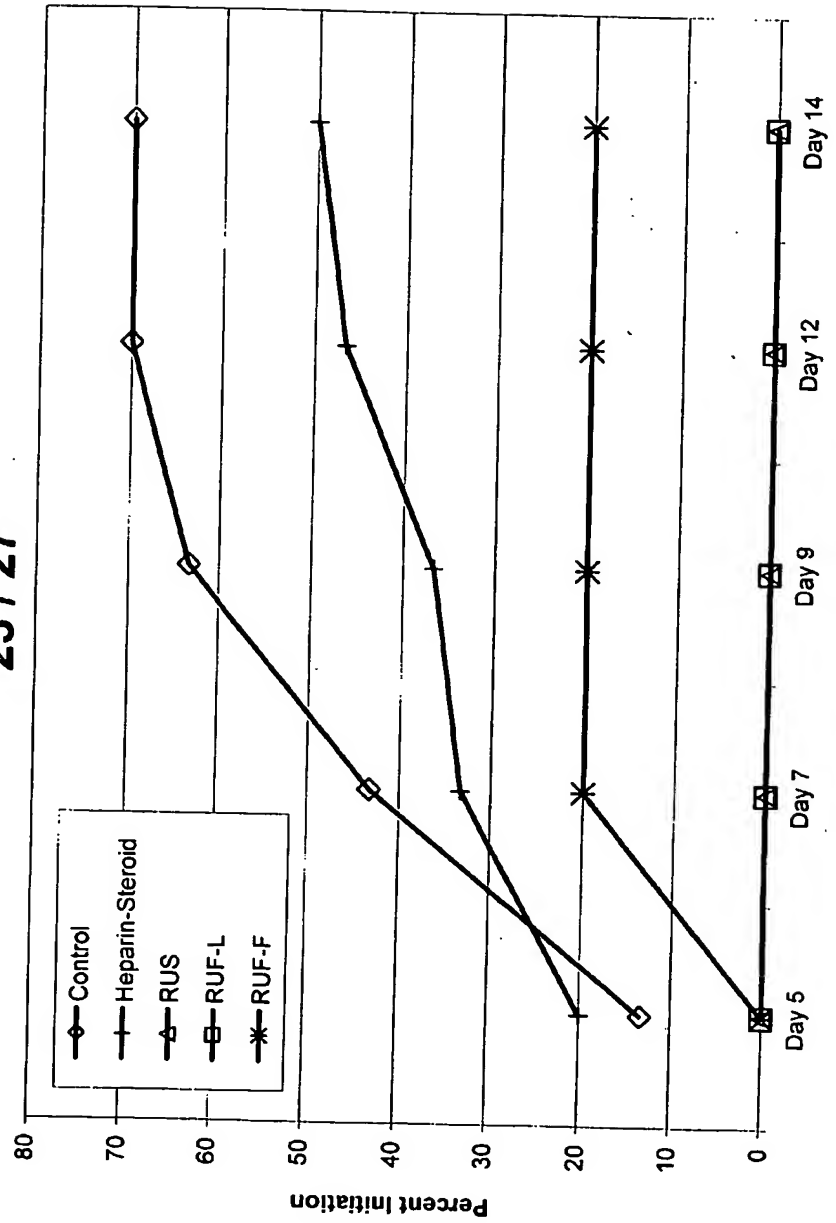

Fig. 11A

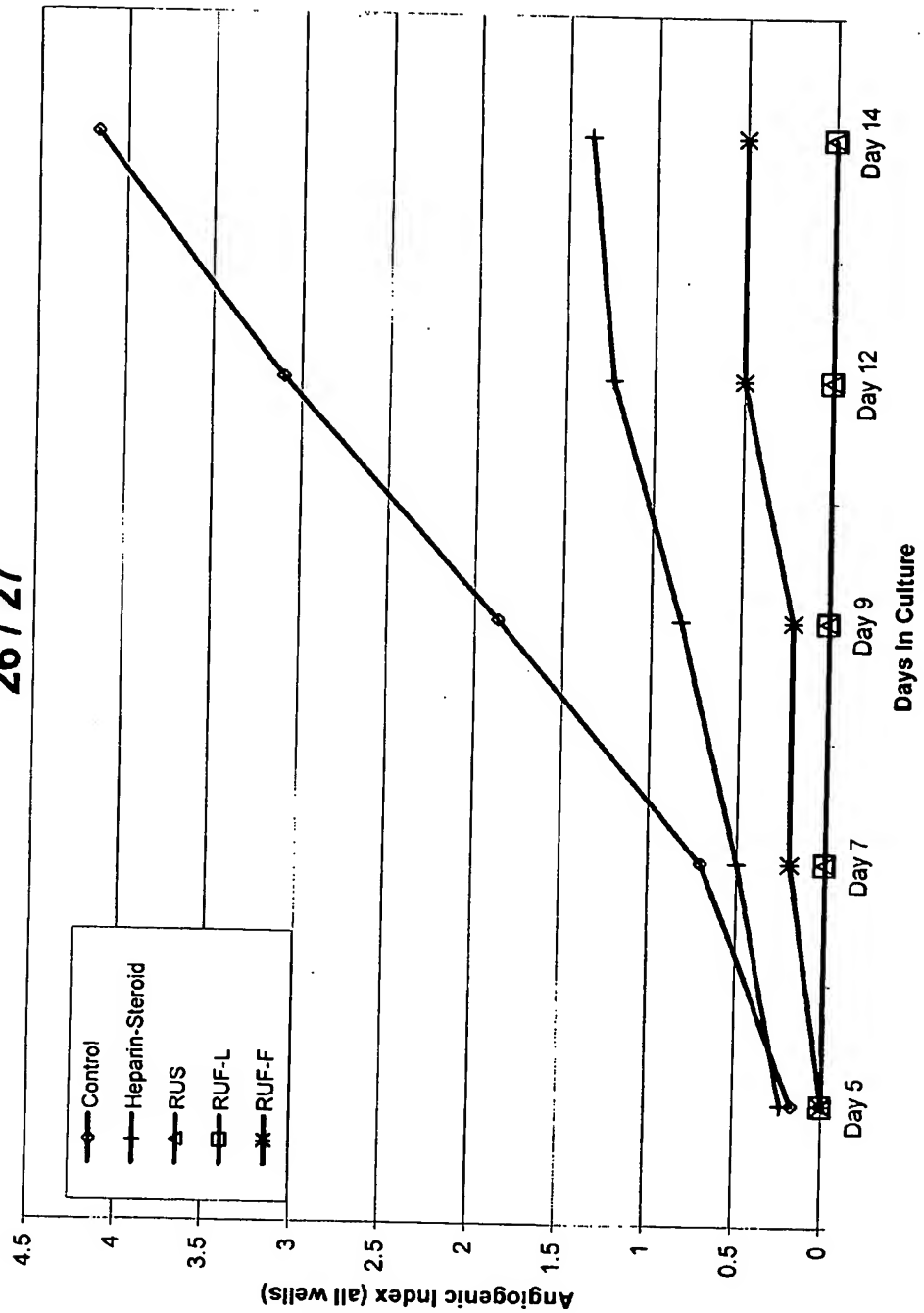

Fig. 11B

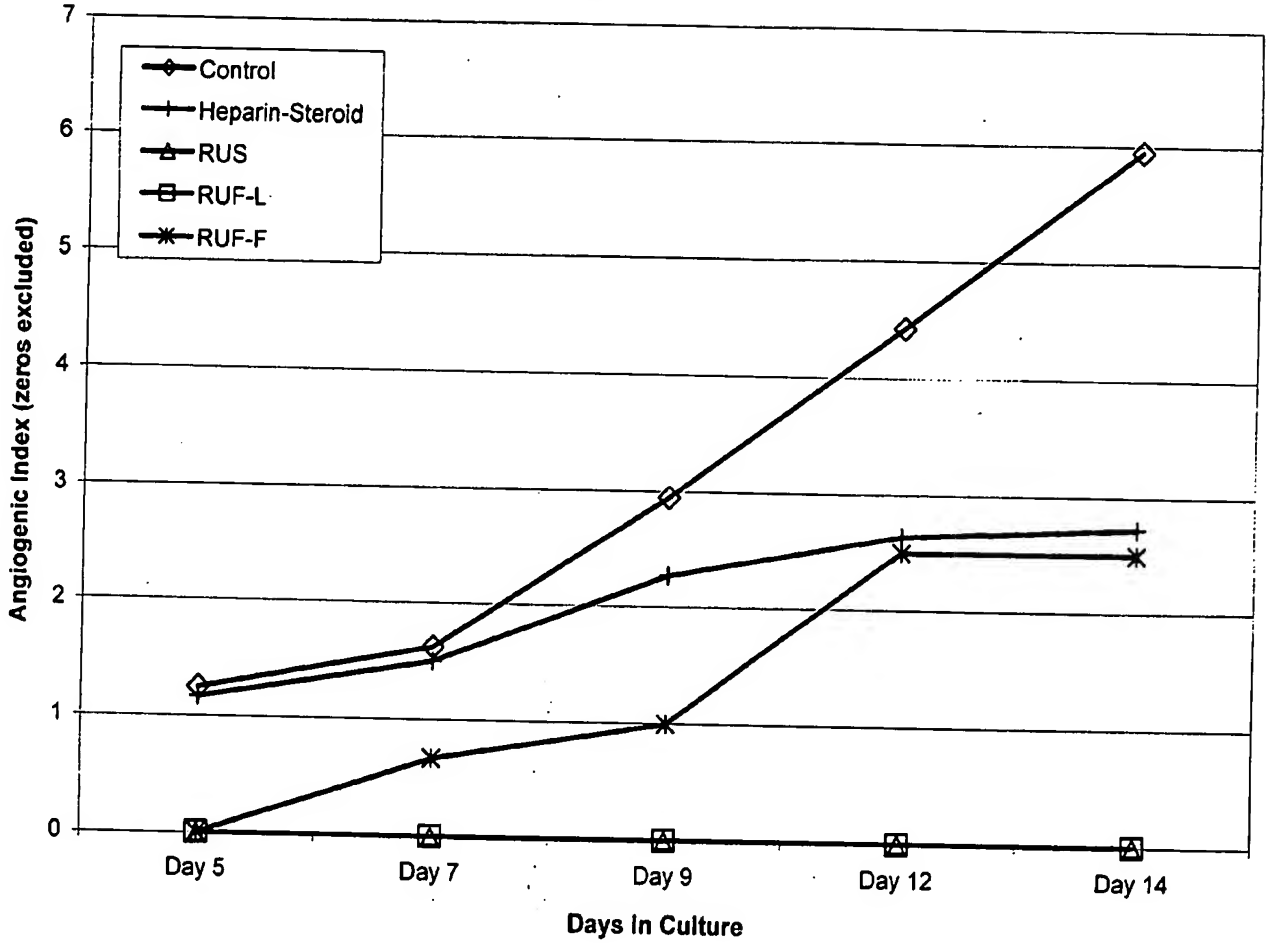

Fig. 11C